RAPID PRESUMPTIVE IDENTIFICATION OF YEAST IN MEAT PRODUCTS

by

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INTRODUCTION

Yeast is one of the most important microorganisms related to human food resources. It contributes greatly to the food industry in areas such as wine making, single cell protein, beer industry, baking, vitamin production, etc. (Harrison, 1970). However, under special conditions it can act as a potential spoilage organism in food, especially in processed, preserved, and refrigerated food (Walker and Ayres, 1970; Anderson, 1975; Miller, 1979; Comi and Cantoni, 1985; Lin and Fung, 1987). Enumeration and identification of yeast from foods are of great importance in understanding the role of this organism in various food systems. The conventional methods for identification of yeast are time consuming and cumbersome (van Walt and Yarrow, 1984). Sophisticated genetical and biochemical methods have been tested but as yet not completely satisfactory for routine identification of yeasts (Deak and Beuchat, 1987). The increasing number of species being described and the frequent name changes along with many synonyms also make study of yeast taxonomy difficulty and confusing.

Since physiological attributes of different yeast may determine the spoilage potential for various foods, it is important for food scientists to be able to ascertain the general profile as well as the dominate species of yeast populations in foods. Ideally, a good yeast identification

systems should be able to discriminate a wide range of species using minimum labor, material and time. Unfortunately, such a system has not been available yet. There are several ready-made identification kits or instrumental systems for clinically important yeasts. However, they perform reliably for only a very restricted domain of genera and species. Hence, there is a need for developing a new method or system for rapidly isolation and identification yeasts from various food systems.

Although yeast is normally not a major concern to most producers and processors of meats, under conditions such as drying, curing, freezing, and using preservative agents, yeast could compete effectively with bacteria and become the dominant microflora (Pitt, 1974; Dalton et al., 1984; Johannsen et al., 1984; Comi and Cantoni, 1985; Monte et al., 1986). A knowledge of how certain yeasts colonize meat products and the effects of the yeast on the meat products is essential in order to prevent economic losses during spoilage or to maximize desirable fermentations of certain cured meat products by yeasts (Pestka, 1986).

Dyes have long been used as an important selective and differential agent in bacteriological media. However, the triarylmethane group of dyes was more efficiently tested and used (Kligler, 1917; Fairbrother and Renshaw, 1922; Moats and Mallox, 1978). More than half of the commercial differential dye containing media for isolation of clinically important

microorganisms are made of triarylmethane dyes (Lin, 1986). Among 186 kinds of triarylmethane dyes (C.I. 42000 - 44999) (Color Index, 1971), most of them are still unknown for their antimicrobial and differentiational properties, especially for the yeasts.

The concepts and applications of many miniaturized microbiological techniques were reviewed by Hartman (1968). Rapid and miniaturized methods were developed to save time, space, labor and material in doing microbial experiments. Compared to the commercial identification kits and automation instruments in microbiology, Fung's miniaturized system (Fung and Hartman, 1975) is the least expensive and the most flexible one (Calvo, 1985). Since Fung's miniaturized system can generate numerous data in a short time, the data interpretation by computer assistance is necessary.

The purpose of this study was to develop rapid methods for the identification of yeasts in food systems, emphasizing on the meat products by using new dye containing media (concentrating on triarylmethane dyes) and Fung's miniaturized system in conjunction with computer.

LITERATURE REVIEW

I. Yeast Identification

Yeast is a unicellular fungus that reproduces by budding or fission (Kreger-van Rij, 1984), which is traditionally characterized, classified, and defined by morphological and physiological criteria (e.g. shape of cells, modes of sexual and asexual reproduction, anaerobic fermentation and aerobic assimilation of sugars and certain growth requirements). In addition to conventional diagnostic tests, biochemical methods provide important data for characterization of yeast. Similarities or differences in the important macromolecules in cells (DNA, RNA, proteins, and polysaccharides) can be used to elucidate not only the degree of relatedness but also to reveal the evolutionary connections (Deak and Beuchat, 1987). However, in routine practice, identification of yeasts relies upon the morphological and physiological features which can be determined by classical, conventional procedures and commercial identification kits or systems. Comparison of unknown strains with descriptions of recognized species can be achieved by identification keys, tables, punch cards, and computers (Barnett et al., 1983; Kreger-van Rij, 1984). Therefore, sophisticated biochemical methods, classical conventional procedures, and rapid commercial systems are three categories described in the following sections for yeast identification.

(1) Sophisticated Biochemical Methods

Along with the development of biochemistry and instrumentation, more and more advanced sophisticated biochemical tests are used for characterization of microorganisms. Extensive investigations of 16S ribosomal RNA (rRNA) have revolutionized our conception of kingdoms of living organisms (Woese and Fox, 1977) and radically changed the taxonomy of bacteria (Fox et al., 1980; Schleifer and Stackebrandt, 1983). A similar method of molecular phylogeny concerns the 5S rRNA of eucaryotic organisms with data accumulating for fungi (Walker, 1985; Walker and Doolittle, 1983). However, there are not enough data available on 5S rRNA sequences of yeast for a comprehensive treatment of higher taxa although some other methods of molecular biology have been applied for decades and provided important clues for taxonomical considerations on the genus and species level (Meyer and Phaff, 1972; Price et al., 1978). It is generally accepted that any difference in the guanine plus cytosine (G+C) base composition of DNA greater than 1.5 to 2.0 mol% excludes the possibility that two strains belong to the same species (Price et al., 1978). Nevertheless, similar G+C ratios do not necessarily provide evidence of species identity. However the identity can be confirmed by the sequence homology (complementarity) between DNA molecules, which is generally higher than 60% to 80% between DNA extracted from strains of the same species (Price et al., 1978; Kurtzman, 1984).

Much research has been devoted to determining the chemical composition of cell wall of yeasts. Free fatty acids, capsular polysaccharides, whole cell hydrolysates and antigenic determinants can be analyzed to provide valuable information for classification (Arx and Weijman, 1979; Ballou et al., 1974, Gorin and Spancer, 1970; Phaff, 1971; Tsuchiya et al., 1974) . However, investigation of electrophoretic patterns of enzymes has been of less value, although coenzyme Q analysis showed promising results for characterizing yeast genera (Yamada et al., 1977).

Molecular biology and biochemistry have made a great impact on yeast classification. Two decades ago the genus Saccharomyces encompassed more than 40 species (Lodder, 1970). However, with the results of the determination of DNA base composition and homology, the number was reduced to about 20 species (Bicknell and Douglas, 1970; Price et al., 1978; Yarrow and Nakase, 1975). More recently, the number of recognized Saccharomyces species has been reduced to seven (Yarrow, 1984), but the controversy will continue and suggestions have been made for once again establishing some formerly separate species on the very same basis of DNA relatedness (Martini and Kurtzman, 1985; Rosini et al., 1982).

The genus Candida is a large but heterogenous assemblage of asporogenous (anamorphic, imperfect) yeast with about 200 species , containing more than one-third of the yeast species (Meyer et al., 1984). The data from studies on the composition

of cell wall and whole cell hydrolysates may help to resolve heterogeneity of this genus (Arx and Weijman, 1979).

(2) Conventional Procedures

The conventional procedures for identification of yeasts were described by van der Walt and Yarrow (1984). These procedures rely heavily upon morphological characteristics of sexual reproduction, with physiological characteristics (fermentation and assimilation properties) mainly used for determination of species. Table 1 is the summary of various morphological and physiological tests for yeast identification.

The extensive list of tests used in the standard description may discourage one from identifying a yeast to the species level. However, not all tests have the same distinctive value for all the species. This partly depends on the genus to which the yeast belong. Furthermore, the procedures which lead to determination of genera and species, such as the use of keys and tables, indicate which tests are of primary importance in an identification. Some of these tests are typical for only a few known species. Nevertheless, it is necessary to establish the other tests in the standard the identity of a given yeast with a described species.

The first step in the identification is to establish the genus of an unknown culture. For the ascosporogenous yeasts, characteristics of sexual reproduction are very important and therefore the first step in identification is to induce

Table 1. Various Morphological and Physiological Tests for Yeast ID

I. Cultural characteristics on or in the following media:
- A. Malt extract
- B. Glucose-yeast extract-peptone water
- C. Surface of assimilation media
- D. Malt (extract) agar
- E. Malt agar plus 2% calcium carbonate
- F. Glucose-yeast extract-peptone agar
- G. Morphology agar
- H. Agar medium containing phenolic compounds
- I. Canavanine-glycine-bromothymol blue (CGB) agar

II. Vegetative reproduction characteristics:
- A. Slide & Dalmau plate cultures on potato, corn meal or morphology agar
- B. Formation of asexual endospores, chlamydospores, Germ tubes, Ballistospores

III. Sexual characteristics
- A. Formation of ascospores
- B. Life cycle

IV. Physiological characteristics
- A. Fermentation of carbohydrates
- B. Assimilation of carbohydrates and other carbon compounds
- C. Splitting of arbutin
- D. Assimilation of nitrate, nitrite,ethyl amine HCl, cadaverine.2HCl,creatine
- E. Growth in vitamin-free medium
- F. Growth on 50% (W/W) glucose-yeast extract agar
- G. Growth in 10% sodium chloride plus 5% glucose in yeast nitrogen base
- H. Growth at 37C
- I. Acid production from glucose
- J. Starch formation
- K. Urease
- L. Fat splitting
- M. Formation of esters
- N. Growth in the presence of Cycloheximide
- O. Growth in the presence of 1% acetic acid
- P. Gelatin liquefaction
- Q. Color reaction with diazonium Blue B (DBB)
- R. Coenzyme Q system
- S. G + C

Adapted from van der Walt and Yarrow (1984)

formation of spores. Of equal importance is the examination of the mode of vegetative reproduction. The results of carbohydrate assimilation, carbohydrate fermentation, and other physiological tests can further narrow a culture to a few genera or even one genus. For ballistospores forming yeasts and ascosporogenous yeast, most genera are recognized by unique features of the vegetative reproduction, such as germ tube formation.

Keys and tables of different genera will indicate which tests are required for recognizing the culture to species level. Fermentation and assimilation of carbohydrate by unknown cultures are important tests for yeast identification

Although it is essential to perform the classical conventional procedures to describe a new species or confirm the identity of some yeast culture, it seems unrealistic to use conventional procedures in routine diagnostic procedures. For example, in hospital or in doing extensive ecological studies of the yeasts, too much time, material and labor would be involved in doing all the conventional tests of identification.

(3) Rapid and Automation System

Since some yeasts are among the most common etiological agents encountered in hospital, rapid recognition and identification of these yeasts are essential for patient management (Huppert et al., 1975). Because the conventional

procedures for yeast identification are time consuming, several identification kits or automation systems for performing these diagnostic work have been developed for clinically important yeasts. Although there are more than 500 recognized species, fortunately less than 30 are associated commonly with humans, and fewer than 20 have been established as the etiological agents of disease (Ahearn and Schlitzer, 1981; Kahanpaa, 1971). In order to simplify the identification procedures, Ahearn and Schlitzer (1984) created a key to identify yeasts pathogenic for man and animals. Most commercial yeast identification kits and systems are able to differentiate these 30 yeast species commonly found in humans. Table 2 summarized the yeast taxa database in some commercial yeast identification kits or systems.

All the commercial ready-made identification kits or systems utilized the concept of miniaturization in microbiology. Some commercial identification systems, such as Uni-Yeast Tek (Flow Lab. Inc.,), Vitek System (McDonnell Douglas Health System Co.), Minitek (Becton Dickinson Microbiology Systems), and Quantum II (Abbott Laboratories), adopted the tests which have been conducted in conventional identification procedures. Table 3 showed the diagnostic tests in these four commercial identification systems.

Some other commercial identification systems such as API Yeast-IDENT, utilized both miniaturized conventional and chromogenic tests for the identification of yeast or yeast-

Table 2. Yeast Taxa Database in Commercial ID Kits and Systems

Yeast Taxa	Uni*	Vit*	Min*	YSt*	API	Abb
Candida albicans	+	+	+	+	+	+
C. catenulata	+					
C. ciferrii	+				+	
C. curvata	+					
C. famata	+	+	+	+		+
C. glabrata	+	+	+	+		+
C. guilliermondii	+	+	+	+	+	+
C. humicola	+				+	+
C. ingens	+					
C. intermedia	+					
C. krusei	+	+	+	+	+	+
C. lambica					+	+
C. lipolytica	+	+		+	+	+
C. lusitaniae	+				+	+
C. macedoniensis	+					
C. membranaefaciens	+					
C. parapsilosis	+	+	+	+	+	+
C. paratropicalis					+	
C. pintolopesii		+				
C. pseudotropicalis	+	+	+	+	+	+
C. rugosa	+	+	+		+	+
C. stellatoidea	+	+	+	+	+	+
C. tropicalis	+	+	+	+	+	+
C. utilis	+					+
C. viswanathii	+					
C. zeylanoides	+	+			+	
Cryptococcus albidus	+	+	+	+	+	+
Cry. gastricus	+		+			+
Cry. laurentii	+	+	+	+	+	+
Cry. luteolus	+	+	+			+
Cry. neoformans	+	+	+	+	+	+
Cry. skinneri						+
Cry. terreus	+	+	+		+	+
Cry. uniguttulatus	+	+	+		+	+
Debaryomyces hansenii	+					
Geotrichum candidum	+		+		+*	+*
G. penicillatum	+				+*	+*
Hanseniaspora guilliermondii				+		
H. uvarum					+	
Hansenula anomala	+					+
Kluyveromyces lactis					+	
Kluyveromyces marxianus	+					
Pichia stagnora	+					
P. wickerhamii	+					
P. zopfii	+					
Prototheca stagnora					+	

Table 2. (continued)

Yeasts	Uni	Vit	Min	YSt	API	Abb
P. wickerhamii					+	
P. zopfii					+	
Rhodosporidium glutinis	+	+	+		+	+
R. graminis						+
R. minuta						+
R. pilimanae	+	+			+	+
R. rubra	+	+	+	+	+	+
Saccharomyces cerevisiae	+	+	+	+	+	+
S. salmonicolor		+				
Sporobolomyces salmonicolor					+	
Torulaspora rosei						+
Torulopsis. candida						+
T. glabrtata						+
Trichosporon beigelii	+	+			+	
T. capitatum	+	+		+	+	
T. inkin	+	+	+			
T. penicillatum	+	+		+		
T. pullulans	+	+	+			+
The number of species identified	46	27	26	15	39	33

Note:

* = not specified;

Uni = Uni-Yeast-Tek^{TM}, Flow Laboratories, Inc. 7655 Old Springhouse Rd., Mclean, VA 22102;

Vit = Vitek System, Vitek System, McDonnell Douglas Health Systems Company, 595 Anglum Dr.ve, Hazelwood, MO. 63042;

Min = Minitek^{TM}, Becton Dickinson Microbiology Systems, PO. Box 243, Cockeysville, MD. 21030-0243;

YST = Yeast Star, Lumac, bv, PO Box 31101, 6370Ac, Landgraaf, The Netherlands.

API = API20C, ApI laboratory Products, Ltd. 8114 Trans Canada Highway st. Laurent, Quebec H4S 1M5.

Abb = Quantum II microbiology system, Abbott Diagnostics, Abbott Park Il 60064;

Table 3. Diagnostic Tests in Some Commercial ID Kits and Systems

Test	Uni	Vit	Min	Abb
Sucrose	+	+	+	+
Lactose	+	+	+	+
Maltose	+	+	+	+
Raffinose	+	+	+	+
Cellobiose	+	+	+	+
Soluble starch	+		+	
Trehalose	+	+	+	+
Inositol	+	+	+	+
D-Xylose	+	+	+	+
Dulcitol	+	+	+	+
Galactose		+	+	+
L-Arabinose		+	+	+
Erythritol		+		+
Adonitol		+	+	
Melibiose		+	+	+
Citric acid			+	
Mannitol			+	
Rhamnose			+	+
Glucose		+	+	+
Methyl-glucoside		+		
Melezitose		+		+
N-acetyl-D-glucosamine		+		
Palatinose		+		
Glycerol		+		
Sorbitol		+		
2-Keto-d-gluconate		+		
Nitrate assimilation		+		+
PHBA & protocatechuic acid				+
Cycloheximide		+		
Xylitol		+		
Urea	+	+		+
Glucose fermentation	+			
Sucrose fermentation	+			
Germ tube	+			
37C growth	+			
40C growth	+			
C/N screen	+			
Pseudohyphae	+			
Blastospores	+			
Arthrospores	+			
Chlamydospores	+			
Endospores	+			
Ascospores	+			

Note: + = Test is available in the system.

like organisms. The basic principle of the tests in this system is to detect the presence of specific enzymes, which can hydrolyze some special compounds into two parts, one of these two parts can give a unique color under certain condition (API, 1986).

Sobczak (1985) devised a unique disk-diffuse test for differentiation of yeast species, which now became into a commercial product called 'Yeast Star' (Lumac bv, the Netherlands). This method relies on the inhibitory effect of six special chemicals on the yeast growth. The results could be interpreted by comparing the coded response with the coded chart. There was 95.3% agreements of results performed by Yeast Star and API 20C (Sobczak, 1985).

There are numerous evaluation studies on various commercial yeast identification systems. The agreements of the results obtained from various commercial identification systems and that from conventional methods are between 54-99.2%, with the average of 89.42% (Lin, 1986). However, all the commercial yeast identification systems are for the clinical use, with very restricted domain of genera and species.

II. Effect of Dyes on Microorganisms

Numerous studies have evaluated the inhibitory properties of dyes in microbiological media to facilitate the isolation or identification of microorganisms since the late nineteenth century (Churchman, 1912; Fairbrother and Renshaw, 1922; Graham-Smith, 1919; Kligler, 1917; Krumwiede and Pratt, 1914; Petroff and Gump, 1935; Stark and Curtis, 1936; Stearn and Stearn, 1926; Stoke and Osborne, 1977; Wilcock, 1977; Miller and Banwart, 1965; Moats et al., 1974; Moats and Mallox, 1978; Fung and Miller, 1973; El-Kashef et al., 1983). Existing methods such as the minimum inhibitory concentration (MIC), the zone of inhibition tests (ZOI), and the Fung's system (Fung and Miller, 1973; Lin and Fung, 1985) are commonly used for assessing antimicrobial agents of compounds.

Factors influencing the antimicrobial activity of dyes in bacteriological media include the ionic nature, chemical structure, and concentration of the dye; the composition, pH, and temperature of the growth medium; and the species, ages, etc. of the microorganisms. In general, basic dyes are more inhibitory than acid or nonionic dyes at a given concentration, especially those containing a di- or triarylmethane nucleus (Fairbrother and Renshaw, 1922; Fung and Miller, 1973; Kligler, 1917; Moats and Mallox, 1978). Other chemical classes of dyes may exhibit antimicrobial properties much as acridine, azine (oxazine, safranine, thiazine), pyrazolne, and xanthen (El-Kashef et al., 1983;

Fairbrother and Renshaw, 1922; Fung and Miller, 1973; Kligler, 1917; Moats and Mallox, 1978).

Studies by Joklik and Smith (1972) have shown that both anionic and cationic dyes which possess antimicrobial properties act by modifying or destroying the functional groups of proteins. Anionic dyes react with amino and imidazole groups, and the degree of their antimicrobial activity is in direct proportion with the acidity of the growth medium (Joklik and Smith, 1972; Stearn and Stearn, 1926). According to Fairbrother and Renshaw (1922) and Kligler (1917), the antimicrobial properties of dyes and related compounds are a function of the benzene nucleus, as well as the type and number of added functional groups. For example, alkyl radicals on the amino groups increase the antiseptic power of triarylmethyl or eliminate the bacteriostatic action of the dye molecule. Moats and Maddox (1978) have postulated that the number of negatively charged groups on bacterial cells increase pH which increases the binding of positively charged dye molecules.

Microorganisms vary in their resistance to a specific dye. Generally, gram positive bacteria are more sensitive to dyes than gram negative bacteria, even though there are considerable variabilities with these two groups of microorganisms (Churchman, 1912; Krumwiede and Pratt, 1914; Moats and Mallox, 1978; Stearn and Stearn, 1926).

In commercial dehydrated culture media, dyes are

essential to the preparation of most differential culture media. In these media, the dyes may act as bacteriostatic agents, inhibitors of growth, reduction-oxidation (redox) indicators, or indicators of changes in the degree of acidity or alkalinity of the substrate. Brom phenol blue, Brom cresol green, Brom cresol purple, Brom thymol blue, Thymol blue, Cresol red, Phenol red, Methyl red, Neutral red, and Litmus are usually used in commercial media as pH indicators while Methylene blue, and Resazurin are used as redox indicators. Acid fuchsin, Aniline blue, Basic fuchsin, Brilliant green, China blue, Crystal violet, Ethyl violet, Eosin Y, Malachite green, Methylene blue, Rose bengal, and Trypan blue are commonly used in the differentiation media, by inhibiting either gram positive or gram negative bacteria, coloring special colonies, or being bacteriostatic agents (Lin, 1986). Table 4 summarized some commonly seen dyes in the commercial differential media.

It is important to note that from a selection of thousands of dyes, only twelve kinds are employed in producing commercial differential media. Therefore, there is a good chance to develop some new dye-containing media for the microbial differentiation purposes, especially for yeast isolation and identification.

Table 4. Dyes in the Commercial Differential Media

Dyes	Function	Commercial Media
Acid fuchsin	Color differential for enterics	Hektoen enteric agar
Aniline	Color differential for faecal coliform	M-FC media
Basic fuchsin	Color differential for coliform	Endo agar; Brilliant green bile agar; M-coliform broth; M-Endo broth; M-HD Endo broth; MoEndo agar LES
Brilliant green	Inhibit G (+)	Bismuth sulfite agar Brilliant green agar Brilliant green bile agar Brilliant green bile broth EE broth Mossel M-Brilliant green broth M-Brilliant green agar M-Bismuth sulfite broth SBG agar SBG sulfate broth *Salmonella Shigella* agar Tetrathionate broth
Crystal violet	Color differential for *Staphylococcus*	Crystal violet agar
	Inhibit G (+)	Crystal violet lactose broth Conradi Drigalske agar
	Suppress G(+) for *Mycobacteria*	Dorset egg medium
	Suppress G(+) for *Streptococcus*	Edwards medium
	Suppress G(+)&G(-)	Littman (oxgall) agar Mitis Salivarius agar Pike *Streptococcal* broth PPLO broth Streptosel agar

Table 4. (continued)

Dyes	Function	Commercial Media
	Inhibit G(+)	Maconkey media Sorbitol agar Violet peptone bile lact. Violet red bile agar Yersinia selective agar
Eosin Y	Color differential for enterics	EMB agar M-EMB agar
Malachite green	Bacteriostatic agents	Acid egg medium ATS medium; IVT medium; L-J medium; Middlebrook 7H10 agar Middlebrook Dubos medium; Petragnani medium Pyruvic acid egg medium
Methylene blue	Color differential for enterics	EMB agar M-EMB broth
Rose bengal	Suppress mold growth	Cooke Rose bengal agar Rose bengal agar
Trypan blue	Assist in colony recognition	Chlamydospore agar
	Color differential for Streptococcus	Mitis Salivarius agar

Adapted from Lin (1986).

III. Food-borne Yeasts in Meat Products

According to Kreger-van Rij (1984), there are 60 genera and 500 species of yeast. Food-borne yeast accounts for about 220 species in 43 genera (Deck and Beuchat, 1987). This is because various foods provide extremely wide ecological environments for yeasts. Table 5 summarized the distribution of food-borne yeasts in various food systems. In this review, the emphasis was made on the food-borne yeasts in meat products.

(1) Distribution and Impact

Much research works have been done in the study of yeast flora in various meat products. Dalton et al. (1984) isolated and identified 383 yeasts in a comparative study of yeast flora between British fresh sausage and minced beef. The majority yeast genera were Candida, Cryptococcus, Debaryomyces, Pichia, Rhodotorula, and Torulopsis. They found that Debaryomyces hansenii was the most commonly isolated yeast from most samples, followed by Candida zeylanoides and Pichia membranaefaciens. The sulphite in sausages did not appear to affect the numbers and kinds of yeast present but did affect their relative proportions.

Monte et al. (1986) studied the fungal profiles of Spanish country-cured hams. From 160 surface samples of 40 hams, yeast counts were between 10^4 and $3X10^5$ Colony Forming Units(CUF)/g and filamentous fungi counts were from $5X10^2$ to $3X10^4$ CFU/g. Debaryomyces marama isolated from these samples

Table 5. Food-borne Yeasts in Various Foods

Genera	# of species	Food System
Arthroascus	1	I
Brettanomyces	6	D,E,F
Bullera	4	A,G,H,J
Candida	73	A,B,C,D,E,F,G,H,I,J
Citeromyces	1	A,B,C,E
Clavispora	1	A,D
Cryptococcus	13	A,B,C,D,E,F,G,H,I,J
Debaryomyces	4	A,B,C,D,E,F,G,H,I,J
Dekkera	3	A,D,E,F
Eeniella	1	F
Endomyces	1	E,F,I
Endomycopseisa	1	E
Filobasidiella	1	A,E
Filobasidium	2	A,E,F,G
Geotrichum	4	A,E,F,G,H.I
Hanseniaspora	6	A,B,D,E,F
Hyphopichia	1	A,B,E,I
Issatchenkia	2	A,B,C,D,E,F,G,H,I
Kluyveromyces	3	A,C,D,E,F,H,I
Leucosporidium	1	A,E,G
Lipomyces	1	J
Lodderomyces	1	C,D,G
Metschnikowia	2	A,C,D,E,G
Nadsonia	1	J
Pachysolen	1	J
Pichia	31	A,B,C,D,E,F,G,H,I,J
Rhodosporidium	2	A,G,I
Rhodotorula	7	A,B,CD,E,F,G,H,I,J
Saccharomyces	6	A,B,C,D,E,F,G,H,I.J
Saccharomycodes	1	A,E
Saccharomycopsis	1	I
Schizosaccharomyces	4	A,C,D,E,F,I
Schwanniomyces	1	J
Sporidiobolus	1	A,E,G
Stephanoascus	1	G
Sterigmatomyces	1	H
Torulaspora	2	A,B,C,D,E,F,G,H,I,J
Trichosporon	3	A,E,F,G,H,I
Trigonopsis	1	E,F
Wickerhamiella	1	A,C,E
Williopsis	4	A,D,E,G,I,J
Zygosaccharomyces	8	A,B,C,D,E,F,H,I

Note: The information is from Deck and Beuchat (1987)
A=plant material; B=fermented/preserved;
C=concentrated sugar; D=beverages; F=beer; G=meats;
H=dairy; I=miscellaneous; J=environment.

could grow at 16% NaCl. Various filamentous fungi, such as Eurotium repens, Penicillium expansum, P. cyclopium, P. viridicatum, P. brevicompactum and P. simplicissimum were also identified.

Comi and Cantoni (1985) researched on total yeast count from 150 samples of fresh and refrigerated meat obtained from various locations in Italy. They found that most fresh samples had a yeast count of 0-100 CFU/g, with 80% in the range 0-10^3 CFU/g. 60% of samples stored under refrigeration for 7 days had counts of 10^5-10^6/g; after 14 days 60% had 10^6-10^7 CFU/g. To study growth of yeasts during refrigerated storage (2C,82%RH), 40 meat samples were classified into 5 groups, depending on initial count level, and stored for 18 days, with yeast count determined every 3 days. The results of composition of yeast flora on fresh and refrigerated meat samples revealed that Torulopsis spp. were predominated (35% of total) on fresh meat, followed by Trichosporon (25%). After 7 days of refrigerated storage, Trichosporon spp. predominated (45%), followed by Candida spp. (20%). Debaryomyces hansenii, Endomycopsis platypodys and Lipomyces starckey, were present on fresh samples, but absent on refrigerated samples. Rhodotorula spp. and Cryptococcus spp. increased on refrigerated samples.

Hsieh and Jay (1984) characterized and identified 194 yeasts isolated from 28 samples of fresh and 4 of spoiled ground beef. 79 strains were from five genera with the genus

Candida accounting for 82% of the strains and 61% of the identified species. Other genera found were Rhodotorula, Torulopsis, Trichosporon, and Cryptococcus. Candida lipolytica were the most frequently isolated species in their study and C. zeylanoides was more indigenous to ground beef than any of the other 21 species identified.

Johannsen et al. (1984) examined the yeast flora present in minced beef before and after radurisation. No reduction in the number of yeast was observed after the meat was radurised at a dose of 2.5KGy. A definite increase in the number of psychrotrophic yeast was observed in radurised meat after 14 days of storage at 4C. The recovered yeast flora comprised representatives of the following species: Candida famata (9 isolates), Cryptococcus albidus (6 isolates), Cry. infirmo-miniatus (1 isolate), Cry. laurentii (1 isolate), Trichosporon cutaneum (1 isolate), Tr. pullulans (2 isolates), Rhodotorula minuta (1 isolate), and Rh. rubra(1 isolate).

Lowry and Gill (1984) studied the yeast flora on frozen lamb stored at -5 C. Lamb loins wrapped in gas-permeable plastics film and stored at -5 C could developed a yeast flora with maximum number (approximately 10^6CFU/cm^2) after 20 weeks. Yeast isolates were identified as Cryptococcus laurentii, Cry. infirmo-miniatus, Trichosporon pullulans and Candida zeylanoides. No microbial growth was detected on lamb loins stored at -10 C for 40 weeks.

Lin and Fung (1987) isolated and identified yeast from

various foods. They isolated Candida lipolytica (16 isolates), C. zeylanoides (6), Rhodotorula rubra (1) from beef; C. azyma (4), C. famata (5), C. lipolytica (9) from ham; C. famata (1), C. lipolytica (1) from hot dog; C. famata (2), C. lipolytica (9) and Rh. rubra (2) from turkey ham.

Researches also were made on the effect of yeast on the sensory and quality of meat products. Comi et al. (1983b) determined the lipolytic enzyme and esterase activity of yeasts from raw ham on various substrate. The highest lipolytic activity was shown by Torulopsis spp., Trichosporon cutaneum and an unidentified Trichosporon spp. For most species, lipolytic activity of the yeasts was generally less than that of Lactobacillus and Micrococcus. The results revealed that yeasts did not present a major problem in relation to lipolysis in raw ham. However, in another report made by Comi et al. (1983a), 2 endopeptidase from Torulopsis spp. isolated from raw ham were observed. The results shown that the enzyme activity was related with the concentration of NaCl.

Winger and Lowry (1983) made a sensory evaluation of lamb after growth of yeasts at -5 C. Cryptococcus laurentii was inoculated at various densities onto lamb loins, which were then frozen and stored at -5 C for 10 weeks. During storage, yeast numbers increased by 2 log cycles. No foreign flavors associated with high yeast numbers could be discriminated by a trained taste panel.

In the study conducted by Kobatake and Kurata (1983), proteolytic and/or lipolytic yeast species were widely distributed among the genera Candida, Cryptococcus, Debaryomyces, Leucoporidium, Rhodotorula, and Trichosporon. All the yeasts tested were isolated from chilled household foods and raw seafood.

Nwahakwu and Akpata (1987) studied the utilization of carbohydrate and protein by Candida famata during spoilage of snail meat. the results indicated that C. famata was a potential spoilage organism for the snail meat. The shelf life of snail meat at room temperature could be increased by eliminating C. famata.

Table 6. Food-borne Yeasts in Various Meat Products

Meat Products	Yeasts:
Ground or minced beef:	Bullera alba, B. tasugae, Candida albicans, C.blankii, C.buffonii, C. ciferrii, C. curvata, C. famata, C. foliorum, C. glaebosa, C. humicola, C. iberica, C. ingens, C. insectamans, C. lambica, C.lipolytica, C. mesenterica, C.parapsilosis, C. ravautii, C. rugosa, C. sake, C. silvae, C. valida, C. vini, C. zeylanoides, Cryptococcus albidus, Cr. hungaricus, Cr. infirmo-miniatus, Cr. laurentii, Cr. macerans, Cr. skinneri, Cr. uniguttulatus, Debaryomyces hansenii, De. marama, Leucosporidium capsuligenum, Le. scottii, Pichia etchellsae, P. media, P. membranaefaciens, Rhodotorula glutinis, R.graminis, R. rubra, Torulopsis candida, T. domercqii, T. inconspicua, T. norvegica, T. silvatica, T. vanderwaltii, Trichosporon cutaneum, Tr. pullulans and Zygosaccharomyces rouxii
Fresh sauage:	Bullera tsugae, Candida alba, C. ciferrii, C. curvata, C. foliorum, C. humicola, C. ingens, C. lipolytica, C. mesenterica, C. rugosa C. sake C. silvae C. valida C. vini, C. zeylanoides Cryptococcus albidus Cr. hungaricus Cr. laurentii, Cr. macerans, Cr. skinneri, Cr. uniguttulatus, Debaryomyces hansenii De. marama , Leucosporidium capsuligenum, Le. scottii, Pichia etchelsii, P. media, P. membranaefaciens, P.vini, Rhodotorula graminis, R. glutinis, Torulopsis candida, T. domercqii, T. inconspicua, T. norveigca, T. vanderwaltii, T. versatilis and Trichosporon cutaneum
Hot dog :	Candida azyma, C.famata, C.lipolytica, C.zeylanoides, De. hansenii, De. kloeckeri, De. marama, De. nicotiamae, De. subgobosus, T. candida, T. gropengiesseri and T. pullulans
Processed poultry :	Candida intermedia, C.krusei, C.parapsilosis, C.pelliculosa, C. rugosa, C.scottii, Rhodotorula minuta, R. auranticala, R. mucilaginosa, R. glutinis, S. cerevisiae, S. dairensis, Torulopsis holmii, T. albida, T. candida T. famata

Table 6 (continued)

Meat Products	Yeasts
Hams :	C.azyma, C. famata, C.lipolytica and De. marama
Lamb loins:	Candida zeylanoides, Cryptococcus infirmo-miniatus, Cr. laurentii and Trichosporon pullulans
Snail meat:	Candida famata

Compiled from the following references:

Comi,1985; Dalton et al., 1984; Deak and Beuchat, 1987; Hsieh and Jay, 1984; Johannsen et al., 1984; Lin and Fung, 1987; Lowery and Gill, 1984; Monte et al., 1986; Nwahukwu and Akpata, 1987; Walker and Ayres, 1970.

(2) Taxonomy

Through an intensive literature survey, Table 6 was generated to report all food-borne yeast associated with meat products. Because of the frequent change of names, numerous synonyms, and the different views by authors, it is necessary to accommodate the yeast listed in Table 6 in an acceptable system. Table 7 and Table 8 were made according to Kreger-van Rij (1984). Table 7 gave the names of yeasts from meat products as well as their synonyms and the pages in which the standard description were in Kreger-van Rij (1984). Table 8 was made by the practical purpose of giving system classification of food-borne yeast in meat products according to Kreger-van Rij (1984). The names of families and genera, and the number of species in each genus were given.

The following citations were made from Kreger-van Rij (1984) and the emphasis was centered on those related to food-borne yeast in meat products.

i. Ascosporogenous Yeast

Family Saccharomycetaceae: Mycelium, pseudomycelium, arthospores and budding cells side by side or alone. Vegetative reproduction by fission or by budding. Asci may arise after isogamous or heterogamous conjugation; often a stage of vegetative reproduction between diploidization and ascus formation. Ascospores of various shapes, but not needle-shaped. Besides an oxidative dissimilation a fermentative dissimilation is often present.

Subfamily Saccharomycetoideae: mycelium and budding cells, pseudomycelium and (or) single budding cells; vegetative reproduction by fission and budding, or budding only; conjugation may or may not immediately precede ascus formation, ascospores of various shapes; dissimilation from merely oxidative to predominantly fermentative.

Genus Debaryomyces: Multilateral budding. Asci conjugated, usually mother-daughter cell conjugation. Ascospores spherical or oval, warty or with ridges, not liberated. Fermentation absent or present, not vigorous; nitrate not assimilated.

Genus Hansenula : Multilateral budding; pseudo- or true mycelium may be present. Ascospores hat-, Saturn-shaped or hemispherical, generally liberated. Fermentation present or absent; nitrate assimilated.

Genus Issatchenkia: Multilateral budding; pseudomycelium. Asci unconjugated ascospores spherical and warty, not liberated. Pellicle formation on liquid media. Fermentation, nitrate not assimilated.

Genus Pichia: Multilateral budding, often pseudomycelium, true hyphae if present, usually scarce, exceptionally abundant arthrospores may be formed. Asci conjugated or unconjugated, they are single yeast cells or pseudomycelial cells; ascospores spherical, hemispherical, hat- or Saturn-shaped, smooth or warty, generally liberated. Fermentation present or absent; nitrate not assimilated.

Table 7. Food-borne Yeast in Meat Products Described by Kreger-van Rij (1984)

Names	Synonym	Page Numbers*
Bullera alba		578-579
B. tsugae		583-594
Candida albicans		609-613
C. azyma		627-628
C. blankii		633-634
C. bufonii		640-641
C. catenulata		648-650
C. ciferrii	Stephanoscus ciferrii	431-433
C. curvata		657-658
C. diddensiae		665-667
C. etchellsii		674-675
C. famata		675-677
C. fennica		677-678
C. foliorum		680-681
C. glabrata		687-688
C. glacbosa		688-689
C. gropengiesseri		691-692
C. holmii	T. holmii	699-700
C. humicola		703-703
C. iberica	C. zeylanoides	839
C. inconspicua		708-709
C. intermedia		716-718
C. ingens		710-711
C. insectamans		714-715
C. lambica		729-731
C. lipolytica	Saccharomycopsis lipolytica	408
C. membranaefaciens		742-744
C. mesenterica		744-745
C. norvegica		760-761
C. parapsilosis		765
C. pelliculosa	H. anomala	173
C. pinus		733
C. ravautii	C. catenulata	648-650
C. rugosa		785
C. sake		787-789
C. silvae		796-797
C. silvatica		798-799
C. silvicultrix		799-800
C. tropicalis		818-821
C. valida		824-826
C. vanderwaltii		826-827
C. versatilis		831-832
C. vini		834-835
C. zeylanoides		839-841
Cryptococcus albidus		850-852

Table 7 (continued)

Names	Synonyms	Page Numbers*
Cry. hungaricus		858-859
Cry. infirmo-miniatus	Rhodosp. infirmo-miniatum	520
Cry. laurentii		860-861
Cry. macerans		862-863
Cry. skinneri		868
Cry. uniguttulatus		870
Debaryomyces castellii		132-133
De. hansenii		134-137
De. kloeckeri	De. hansenii	134
De. marama		137-138
De. nicotianae	De. hansenii	134
De. polymorphus		139-140
De. subglobosus	De. hansenii	134
Filobasidium uniguttulatum		488-499
Hansenula californica		180-181
Issatchenkia orientalis		217-219
Leucosporidium scottii		504-506
Metschnikowia pulcherrima		273-275
Pichia anomala		
P. carsonii		317-319
P. etchelsii		323-324
P. fermentans		326-328
P. guilliermondii		329-330
P. haplophiola		330-331
P. humboldii		334-335
P. media		337-338
P. membranaefaciens		338-340
P. rhodanensis		360-361
Rhodosporidium infirmo-miniatum		520-523
Rhodotorula glutinis		897-899
R. graminis		899-900
R. minuta		901-902
R. mucilaginosa	R. rubra	902-903
R. rubra		902-903
Saccharomyces cerevisiae		382-386
S. dairensis		386-387
S. exigus		387-388
S. telluris		391-392
Saccharomycopsis lipolytica		406-408
Sporidiobolus pararoseus		535-537
Sporobolomyces albo-rubesceus		912-913
Spo. puniceus		916-917
Spo. roseus		917-919
Stephanoascus ciferrii		431-433
Torulopsis candida	C. famata	675-677
Tor. domercqii	Wickerhamiella domercqii	443-445

Table 7. (continued)

Names	Synonyms	Page Numbers*
Tor. inconspicua	C. inconspicua	708-709
Tor. norvegica		
Tor. silvatica	C. silvatica	798-799
Tor. vanderwaltii	C. vanderwaltii	826-827
Trichosporon cutaneum		940-946
Tri. pullulans		954-956
Wickerhamiella domercqii		443-445
Williopsis californica	Hansenula californica	180-181
Yarrowia lipolytica	Saccharoymcopsis lipolytica	406
Zygosaccharomyces rouxii		462-465

* These are the page numbers of the book by Kreger-van Rij (1984) for particular yeast cited.

Table 8. Classification of Food-borne Yeast in Meat Products*

Family or Subfamily	Genera	Number of species
Ascosporogenous yeasts:		
Saccharomycetaceae		
Saccharomycatoideae	Debaryomyces	7
	Hansenula	1
	Issatchenkia	1
	Pichia	10
	Saccharomyces	4
	Stephanoascus	1
	Wickerhamiella	1
	Zygosaccharomyces	1
Basidiosporogenous yeasts:		
Filobasidiaceae	Filobasidium	1
Teliospore-forming yeasts	Leucosporidium	1
	Rhodosporidium	1
	Sporidiobolus	1
Imperfect yeasts:		
Cryptococcaceae	Candida	42
	Cryptococcus	6
	Rhodotorula	5
	Trichosporon	2
Sporobolomycetaceae	Bullera	2
	Sporobolomyces	3

* According to Kreger-van Rij (1984).

Genus Saccharomyces: Multilateral budding, pseudomycelium may be formed. asci unconjugated; ascospores spherical or oval, smooth, seldom warty, not liberated. No pellicle formation on liquid media. Fermentation vigorous; nitrate not assimilated.

Genus Stephanoascus: Budding yeast cells, abundant true mycelium, pseudomycelium, no arthrospores. Hyphal septa with plasmodesmata.

Genus Wickerhamiella: Multilateral budding, no pseudomycelium. Asci conjugated; ascospores lentiform, light brown, not liberated. Fermentation; nitrate not assimilated.

Genus Zygosaccharomyces: Multilateral budding; pseudomycelium may be formed. Conjugated asci; ascospores spherical or ellipsoidal, not liberated. Fermentation vigorous; nitrate not assimilated.

ii. Basidiosporogenous Yeast

Family Filobasidiaceae, type species: Filobasidium floriforme Basikia arising in loose or dense groups from a mycelium with clamp connections, slender, non-septate, bearing sessile thin-walled basidiospores terminally; thick-walled probasidia lacking, blastospores present.

Genus Filobasidium: Budding yeast cells; pseudomycelium may occur, true mycelium with clamp connections. Hyaline cultures. Sessile basidiospores in a whorl on the apex of the basidium.

Teliospore-forming yeasts:

Genus Leucosporidium: Budding yeast cells, pseudomycelium may occur, true mycelium with and without clamp connections. Hyaline cultures. No ballistospores. Teliospores. Metabasidia with basidiospores. Fermentation may occur; nitrate is assimilated.

Genus Rhodosporidium: Budding yeast cells; pseudomycelium may occur, true mycelium with and without clamp connections. Yellow, orange or red cultures due to carotenoid pigments. No ballistospores. Teliospores. Metabasidia with basidiospores. No fermentation; nitrate is assimilated or not.

Genus Sporidiobolus: Budding yeast cells, pseudomycelium, true mycelium with clamp connections. Pink or red cultures due to carotenoid pigments. Ballistospores. Teliospores. Metabasidia with basidiospores. No fermentation; nitrate is assimilated or not.

iii. Imperfect Yeasts

Family Cryptococccaceae: Budding yeast cells always present; moreover pseudomycelium, true mycelium and arthrospores may be formed. Cells hyaline, or red, orange or yellow due to carotenoid pigments, very seldom brown or black. Dissimilation strictly oxidative and oxidative and fermentative.

Genus Candida: Multilateral budding, polar budding if present on a narrow base; pseudo-, and true mycelium may be formed. Budding cells not apiculate, flask-shaped or triangular; no formation of sympodia or sterigmata. No strong

acid formation from glucose. Inositol positive strains form pseudomycelium. Visible pigmentation due to carotenoid pigments absent. Fermentation or not; nitrate assimilated or not; inositol assimilated or not.

Genus Cryptococcus: Multilateral budding; pseudomycelium absent or rudimentary. Most strains have capsulated cells; the cultures on solid media are generally mucous; they are hyaline, or red or orange due to carotenoid pigment; in one species brown or black cultures occur under special conditions.

Genus Rhodotorula Harrison: Multilateral budding, pseudo- and true mycelium may occur. The cultures are red or yellow due to carotenoid pigments, the cultures are often mucous. no fermentation, nitrate is assimilated or not, inositol is not assimilated, starch-like compounds are not produced, urease is positive.

Genus Trichosporon: Budding yeast cells, pseudomycelium and abundant true mycelium with arthrospores. Asexual endospores may be formed. Fermentation or not, nitrate is not assimilated.

Family Sporobolomycetaceae: Mycelium, pseudomyucelium and budding yeast cells. Vegetative reproduction by fission or budding. The vegetative cells may form aerial sterigmata that are single or bifurcated. Smooth hyaline spores are formed in an oblique position to the sterigmata. When mature, the spores (ballistospores) are ejected by a drop-excretion

mechanism. Dissimilation strictly oxidative.

Genus Bullera: Budding yeast cells; pseudo- and true mycelium may be formed. The cultures are hyaline. Ballistospores are rotationally symmetrical, spherical, obovoid, turbinate, apiculate or ampulliform. No fermentation, nitrate is assimilated or not, inositol is assimilated or not, starch-like compounds are produced or not.

Genus Sporobolomyces: Budding yeast cells; pseudo- and true mycelium may be formed. The cultures are red or pink due to carotenoid pigments. Ballistospores are bilaterally symmetrical, usually obovoid, pyriform or reniform. No fermentation, nitrate is assimilated or not, inositol is assimilated or not, starch-like compound are produced or not, urease is positive.

(3) A Simplified Identification Key

In order to give a reliable discrimination of the wide range of species of yeast in meat products with less labor, material and time, a simplified key for rapid presumptive identification of food-borne yeast in meat products was proposed, which could differentiate 84 species of meat related yeasts by using 23 physiological tests.

All the physiological attributes of these 84 meat related yeasts were compiled in Appendix 1.

The following principles were used in the process of making the simplified identification key to food-borne yeast

in meat products.

i. Yeast species which only have been found in meat products have been selected;

ii. Diagnostic tests have been reduced by selecting those which have data among all the selected species;

iii. Only three symbols (+, -, v) were used in the description of individual physiological test for each species. The descriptions of 'reaction weakly positive' (+w), 'reaction slow' (+s), and 'the variables' (+) were considered as variable results and recorded as 'v'. While 'reaction positive' (+) and 'reaction positive, seldom negative' (+"-") were recorded as '+'. 'Reaction negative' (-) and 'reaction negative, seldom positive' (-"+") were recorded as '-'. The simplified identification key to food-borne yeasts in meat products is presented in Table 9.

In Table 9, 'v' means variable, that is either positive or negative. Some species have up to ten 'v's, in other words, there are 2^{10} = 1024 kinds of possibilities for these species. Therefore, without the assistance of a computer program, it is impossible to interpret the results for unknown cultures. In this study, a computer program package specially designed for the simplified identification key to food-borne yeast in meat products is donated by Mr. Su Haiping and Mr. Li, Yuangqui,, which is documented in the Appendix 2.

Table 9. A Simplified Identification Key to Food-borne Yeast in Meat Products

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
B. alba	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
B. tsugae	−	−	−	−	−	+	+	+	+	+	−	−	+	+	−	−	+	−	+	+	−	+	−
C. albicans	+	v	−	+	−	+	−	+	−	−	−	−	+	+	−	+	+	v	v	v	−	−	+
C. azyma	−	−	−	−	−	+	+	+	−	−	−	−	+	−	−	−	v	v	+	−	−	−	+
C. blankii	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	+	−	+	+
C. buffonii	−	−	−	−	−	−	−	+	+	−	−	−	+	−	−	+	+	+	−	−	−	−	−
C. catenulata	v	−	−	−	−	+	−	+	−	−	−	−	−	−	−	+	+	−	v	v	−	+	v
C. ciferrii	−	−	−	−	−	+	+	+	+	+	v	+	−	−	+	+	+	+	+	+	+	−	+
C. curvata	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	v	v	v	+	+	v
C. diddensiae	+	−	−	−	−	+	+	+	+	+	−	+	+	−	−	−	+	+	+	+	−	−	+
C. diversa	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	v	−	+	+	−	−	v
C. etchellsii	+	−	−	+	−	+	−	+	−	v	−	−	−	−	−	−	−	−	v	−	−	v	−
C. famata	v	−	v	+	−	+	+	+	+	v	v	v	+	v	+	v	+	+	+	v	−	v	v
C. fennica	+	+	+	+	−	+	+	+	v	+	−	+	+	v	+	+	+	v	+	+	−	+	v
C. foliorum	−	−	−	−	−	−	−	−	+	v	−	−	+	−	−	−	+	v	+	+	−	+	+
C. glabrata	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+
C. glaebosa	−	−	−	−	−	+	+	+	+	−	−	−	+	+	+	+	+	+	+	+	−	−	−
C. gropengiesseri	+	−	+	−	−	+	+	−	+	−	−	−	−	−	+	−	−	−	−	−	−	+	−
C. holmii	+	+	+	−	−	+	+	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	v
C. humicola	−	−	−	−	−	v	+	+	v	+	v	+	+	v	v	v	+	v	v	v	v	v	−
C. inconspicua	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+
C. intermedia	+	+	+	v	−	+	+	+	+	−	v	−	−	+	+	+	+	v	v	v	−	−	−
C. ingens	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	v	−	−	+	v
C. insectamans	+	−	−	−	−	−	−	+	+	+	−	−	+	−	−	−	+	−	+	+	−	−	+
C. lambica	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	+	−	−	+
C. membranaefaciens	+	v	v	−	−	+	+	+	+	+	−	+	+	−	+	−	+	+	v	v	−	+	−
C. norvegica	v	−	−	−	−	−	−	−	+	−	v	−	−	−	−	−	+	v	v	v	−	+	−
C. parapsilosis	+	v	−	−	−	+	+	+	−	v	−	−	+	−	−	−	+	+	v	v	−	−	+
C. pelliculosa	+	v	+	v	−	v	+	+	+	v	−	+	+	−	v	+	v	v	+	+	−	+	v
C. pinus	+	+	+	+	−	+	+	+	+	+	+	−	+	−	+	−	+	−	+	+	−	−	+
C. rugosa	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	v	v	+	v	−	−	+
C. sake	+	+	v	v	−	+	+	+	v	−	−	−	+	−	−	−	+	−	+	v	−	v	−
C. silvae	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	v	−	+	v
C. silvicultrix	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−	−	+
C. tropicalis	+	+	v	+	−	+	v	+	v	v	−	−	+	−	−	−	+	+	+	v	−	−	+
C. valida	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	v	−	−	v	−
C. vanderwaltii	−	−	−	−	−	+	+	−	−	+	−	−	+	−	−	−	+	+	+	+	−	−	v
C. versatilis	+	+	v	−	+	+	v	+	v	v	−	−	+	v	v	−	−	v	−	−	−	+	−
C. vini	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	v	−	−	−	−
C. zeylanoides	−	−	−	−	−	v	−	−	v	−	−	−	+	−	−	−	−	−	+	+	−	v	−
Cry. albidus	−	−	−	−	−	v	+	+	+	v	v	−	+	v	+	v	+	+	+	+	+	−	−
Cry. hungaricus	−	−	−	−	−	+	+	+	+	+	+	−	+	v	+	−	+	+	+	+	+	−	−
Cry. laurentii	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	−	+	+	v	+	+	v	v
Cry. macerans	−	−	−	−	−	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	+	−	−
Cry. skinneri	−	−	−	−	−	+	−	−	+	+	+	−	+	−	−	−	+	+	+	+	+	−	−
Cry. uniguttulatus	−	−	−	−	−	v	+	+	−	−	+	−	+	−	+	−	+	+	+	v	+	+	−
De. castellii	+	−	+	+	−	+	+	+	+	−	+	−	+	+	+	−	+	+	+	+	−	−	−
De. hansenii	+	−	−	−	−	+	+	+	+	v	v	v	−	v	+	+	+	+	+	v	−	−	v

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
De. marama	+	−	−	−	−	+	+	+	+	v	−	+	+	v	+	+	+	+	+	+	−	−	−
De. polymorphus	+	+	+	+	−	+	+	+	+	+	−	+	+	v	+	+	+	v	+	+	−	+	v
Fil. umiguttulatum	−	−	−	−	−	−	+	+	−	−	−	−	+	−	v	+	+	+	−	−	+	−	−
Han. californica	+	−	−	−	−	−	+	v	+	−	v	−	v	−	−	−	+	−	+	v	−	−	−
Iss. orientalis	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	+
Leu. scottii	−	−	−	−	−	v	+	+	+	v	+	−	−	v	+	−	+	v	−	−	−	+	−
Met. pulcherrima	+	−	−	−	−	+	+	+	+	v	−	−	+	−	−	−	+	−	+	v	−	−	v
Pichia carsonii	−	−	−	−	−	+	+	+	v	v	−	−	+	−	v	+	+	v	+	+	−	−	−
P. etchellsii	+	−	−	−	−	+	+	+	+	−	−	−	+	−	−	−	+	+	+	+	−	−	+
P. fermentans	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	+	−	+	+	−	−	+
P. guilliermondii	+	v	+	−	−	+	+	+	+	+	v	−	+	−	+	−	+	+	+	v	−	−	+
P. haplophiola	−	−	−	−	−	+	−	−	−	+	−	+	−	−	−	−	+	+	−	−	−	−	−
P. hunboldii	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	v
P. media	−	−	−	−	−	+	−	+	+	+	−	+	+	−	−	−	+	+	+	+	−	−	−
P. membranaefaciens	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	v	−	v	v	−	v	v
P. rhodanensis	+	−	−	−	−	−	+	+	+	−	+	−	+	−	−	−	+	−	+	+	−	−	+
Rh. infirmo-miniatum	−	−	−	−	−	+	+	+	+	+	v	−	+	+	+	+	+	+	+	+	+	−	v
Rhodotorula glutinis	−	−	−	−	−	+	+	+	+	v	v	−	+	−	+	v	+	v	+	v	−	+	v
R. graminis	−	−	−	−	−	+	+	−	v	v	v	−	−	−	+	−	+	v	+	+	−	+	−
R. rubra	−	−	−	−	−	v	+	v	v	v	v	−	−	−	+	−	+	v	+	v	−	−	v
Sacch. cerevisiae	+	v	v	v	−	v	v	v	−	−	−	−	v	−	v	−	−	−	−	−	−	v	v
Sacch. dairensis	+	+	−	−	−	+	−	−	−	−	−	−	v	−	−	−	−	−	−	−	−	−	+
Sacch. exiguus	+	+	÷	−	−	+	+	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−
Sacch. telluris	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Sa. copsis lipolytica	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	+	+	−	−	−
Sporid. paraoseus	−	−	−	−	−	+	+	+	+	v	−	−	+	−	+	v	v	−	v	v	−	÷	−
Sporo. albo-rubesceus	−	−	−	−	−	+	+	+	−	+	−	−	+	−	+	+	+	+	+	+	−	−	+
Sporo. puniceus	−	−	−	−	−	−	+	+	+	+	−	−	+	−	+	−	−	+	−	−	+	−	−
Sporo. roseus	−	−	−	−	−	v	+	+	+	v	−	−	+	−	+	v	v	v	−	v	−	v	−
Steph. ciferrii	−	−	−	−	−	+	+	+	+	v	−	+	−	−	+	+	+	+	+	+	+	−	+
Torul. delbrueckii	+	v	v	v	−	v	v	v	−	−	−	−	+	−	v	−	−	−	v	−	−	+	−
Torul. globosa	+	−	+	−	−	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	+	+
Tricho. cutaneum	−	−	−	−	−	+	+	+	+	+	v	v	v	+	v	+	+	+	v	v	+	−	v
Tricho. pulluans	−	−	−	−	−	−	+	+	+	v	+	+	+	+	+	+	v	+	+	+	v	+	−
Wicker. domercqii	−	−	−	−	v	+	−	−	+	−	−	−	−	−	−	−	−	−	+	+	−	−	−
Zygosacch. rouxii	+	−	+	v	−	v	v	v	−	+	−	−	v	−	−	−	−	−	−	−	−	v	v

* Notation:

1 = GlF = Glucose fermentation
2 = GaF = galactose fermentation
3 = SuF = Sucrose fermentation
4 = MaF = Maltose fermentation
5 = LaF = Lactose fermentation
6 = Gal = Galactose assimilation
7 = Suc = Sucrose assimilation
8 = Mal = Maltose assimilation
9 = Cel = Cellobiose assimilation
10 = Rib = D-Ribose assimilation
11 = Rha = L-Rhamnose assimilation
12 = Ery = Erythritol assimilation
13 = Tre = Trehalose assimilation
14 = Lac = Lactose assimilation
15 = Raf = Raffinose assimilation
16 = sSt = Soluble starch assimilation
17 = Xyl = D-Xylose assimilation
18 = Ara = L-Arabinose assimilation
19 = SAd = Succinic acid assimilation
20 = CAd = Citric acid assimilation
21 = Ino = Inositol assimilation
22 = Vfr = Growth in vitamin-free medium
23 = 37C = Growth at 37C

MATERIALS AND METHODS

I. Effect of Some Triarylmethane Dyes on the Yeast Growth

This study was designed to determine the effect of some triarylmethane dyes on the growth of food yeasts, with a possible view to make use of the triarylmethane dyes in developing new media for selective isolation and differentiation of specific food yeast species.

(1) Dyes

Twenty kinds of triarylmethane dyes were used in this investigation. The color index number, commercial name, and the corresponding producer of the dyes are listed in Table 10.

(2) Yeast Tested

Seventeen species of Candida, 1 species of Hansenula, 8 species of Saccharomyces, 1 species of Zygosaccharomyces, 3 species of Debaryomyces, 2 species of Pichia, 1 species of Rhodotorula, 2 species of Brettanomyces, 2 species of Trichosporon, and 1 species of Bullera were employed (Table 10). They were maintained on YM agar slants (Difco). All the cultures were grown in YM broth (Difco, pH 6.2; 48 hr at 25 C), and homogenized in sterile water with a Vortex (Fisher) before used as inocula.

(3) Screening Procedures

0.01 gram of dyes was incorporated into 100 ml of the basal agar medium (YM agar, Difco) at final concentration of 1:10,000 dilution before autoclaving. YM agar without dyes was

Table 10. Triarylmethane Dyes Used in Dye-Containing Media Tests

No.	C.I Name	C.I. Name	Commercial Name & Source
1.	44045	Basic blue 26	Basonly blue 640 (BASF)
2	42090	Acid blue 9	Basonly blue NB-757 (BASF)
3.	42000	Basic green 4	Basonly green NB-832 (BASF)
4	42645	Acid blue 15	Basacid blue NB 621 (BASF)
5	42535	Basic violet 1	Flexo violet 600 (BASF)
6	42555	Basic violet 3	Flexo violet 615 (BASF)
7	42760	Solvent blue 23	Neptun blue NB 652 (BASF)
8	42595	Basic blue 7	Basonly blue 638 (BASF)
9	42090	Food blue 2	FD & C blue No. 1 (WJ)
10	42053	Food green 16	FD & C green 3 (WJ)
11	44052	Acid green 16	Intracid green V ex conc (CK)
12	42045	Acid blue 1	Intracid pure blue V ex (CK)
13	42660	Acid blue 83	Inreapel blue R (CK)
14	42090	Acid blue 9	Intracid pure blue L (CK)
15	42640	Acid violet 49	Intracid violet 4BNS ex (CK)
16	42040	Basic green 1	Brilliant green (Sigma)
17	42755	Acid blue 22	Aniline blue (Sigma)
18	42500	Basic blue 9	Pararosaniline.HCl (Sigma)
19	42555	Basic violet 3	Crystal violet (Allied chem.)
20	42510	Basic violet 14	Basic Fuchin (J.T.B.Chem.)

Note: BASF = BASF Corporation Chemicals Division. 491 Columbia Ave., Holland, Michigan 49423.

WJ = Warner & Jenkinsin, Color Division, 2526, Baldwin Street, P.O. Box 14538, St. Louis, Mo. 63178.

Sigma = Sigma Chemical Company, P.O. Box 14508, St. Louis, Mo. 63178.

Allied Chem = Allied Chemical & Dye Corporation, New York, N.Y.

J.T.B.Chem = J.T. Baker Chemical Co. Phillipsburg, N.J.

Table 11. Yeasts Used in the Dye-containing Media Tests

Genera	Species	Source	Stock Code
Brettanomyces	anomalus	NRRL Y-1415	B-1
Brettanomyces	claussenii	NRRL Y-1414	B-2
Bullera	alba	NRRL Y-6655	Bu-1
Candida	albicans	ATCC 10261	C-3
Candida	catenulata	NRRL Y-1508	C-4
Candida	famata	NRRL Y-1453	C-6
Candida	guilliermondii	NRRL Y-7572	C-8
Candida	kruei	ISU HB-2	C-9
Candida	lactis-condensi	NRRL Y-1515	C-10
Candida	lipolytica	ATCC 34088	C-1
Candida	lipolytica	ATCC 20362	C-2
Candida	parapsilosis	NRRL Y-2315	C-14
Candida	parapsilosis	ISU MM-4	C-15
Candida	pseudotropicalis	NRRL Y-1264	C-18
Candida	pulcherrima	ISU HB-29	C-19
Candida	reukaufii	NRRL Y-6343	C-20
Candida	sake	NRRL Y-1622	C-21
Candida	steatolytica	NRRL Y-7136	C-31
Candida	rugosa	NRRL Y-1249	C-30
Candida	tropicalis	NRRL Y-1522	C-25
Candida	utilis	NRRL Y-900	C-26
Candida	vini	NRRL Y-6658	C-27
Debaryomyces	hansenii	NRRL Y-7268	D-1
Debaryomyces	hansenii	NRRL Y-7426	D-2
Debaryomyces	hansenii	NRRL Y-1454	D-3
Debaryomyces	subglobosus	NRRL Y-6666	D-4
Hansenula	anomala	NRRL Y-366	H-1
Pichia	fermentans	NRRL Y-1619	P-1
Pichia	membranaefaciens	NRRL Y-6776	P-2
Rhodotorula	minuta	NRRL Y-1589	R-2
Saccharomyces	bailii	NRRL Y-7253	S-1
Saccharomyces	cerevisiae	NRRL Y-2034	S-5
Saccharomyces	cerevisiae	NRRL Y-12647	S-6
Saccharomyces	chevalieri	NRRL Y-12633	S-7
Saccharomyces	dairensis	NRRL Y-1353	S-8
Saccharomyces	delbrueckii	NRRL Y-1567	S-9
Saccharomyces	globosus	NRRL Y-4099	S-10
Saccharomyces	rosei	NRRL Y-866	S-12
Saccharomyces	rouxii	ISU FY-4	S-13
Trichosporon	beigelii	NRRL Y-1490	T-1
Trichosporon	pullulans	NRRL Y-1522	T-2
Zygosaccharomyces	rouxii	NRRL Y-2547	Z-1

Note: ATCC = American Type Culture Collection (Rockville, MD).
ISU = Iowa State Univ. (Ames, IA).
NRRL = Northern Regional Research Laboratory, Peoria, IL.

used as a positive control. Sterilized media were poured into the disposable petri dishes (Fisher), and kept at room temperature overnight before use. The actively growing broth culture of the tested yeasts were introduced into individual wells of a sterile Microtiter plate to form a master plate. From this master plate, organisms were transferred onto the agar surfaces of the control basal medium and dye-containing media plates by use of a multiple inoculation system (Fung and Miller, 1973). All inoculated plates were incubated at room temperature (21 C), and observed growth of cultures under incandescent and long wave UV light (366nm) every 12 hours up to 10 days. Growth, no growth, morphology, color under incandescent light and fluorescence under long wave light were recorded for each cultures at all sampling time.

II. Effect of Combination of Aniline Blue and Antibiotics on the Growth of Candida albicans and Some Yeast and Bacteria

In the preliminary study, YM agar with Aniline blue at 1:10,000 dilution provided a unique fluorescence under long wave UV light (366nm) for the colony on Candida albicans. In order to study the effect of the combination of Aniline blue and antibiotics of the growth of Candida albicans as well as some yeasts and bacteria, Aniline blue-YM agar was prepared by incorporation of Aniline blue into antibiotics (chlortetracycline.HCl and chloramphenicol, 100 ppm, respectively) containing YM agar at a concentration of 1:10,000 dilution. The antibiotics were added to inhibit bacterial growth in YM agar. The effect of antibiotics on the growth and fluorescence of Candida albicans, Candida lipolytica and Saccharomyces cerevisiae on Aniline blue YM agar was tested in this experiment.

(1) Cultures

All the cultures employed in this study are listed in Table 12. Yeast cultures were maintained in YM agar slant (Difco), and bacteria cultures were maintained on nutrient agar slants (Difco) at temperatures of 21 C and 32 C respectively for 48 hours. One loop of organism was transferred in YM broth (for yeast), or nutrient broth (for bacteria), and homogenized with a Vortex (Fisher) before used as the inocula.

Table 12. Cultures Used in Aniline Blue & Antibiotics Tests

Organisms	Sources
Yeast:	
Candida albicans	ISU 813-12A
Candida albicans	ISU 813-14A
Candida albicans	ATCC 10261
Cadida lipolytica	ATCC 34088
Saccharomyces cerevisiae	Andovin St. ISU
Gram-positive bacteria:	
Staphylococcus aureus	KSU 326
Streptococcus faecalis	ATCC 11454
Leuconostoc mesenteroides	ATCC 10830
Bacillus cereus	UM F-2769
Gram-negative bacteria:	
Escherichia coli	
Enterobacter agglomerans	
Salmonella spp.	
Serratia liquifaciens	
Serratia marcescens	

Note: All the Gram-negative bacteria were provided by Dr. J. S. Bailey, Russell Research Center USDA, Athens, GA.

(2) Media

Four kinds of media were prepared in this study. YM agar was prepared according to the manufacture's instructions; Aniline blue-YM agar was made by adding 0.01 grams of Aniline blue (Sigma) to 100 ml of YM agar; YM agar with antibiotics was prepared by adding antibiotics (100 ppm of chlortetracycline.HCl and 1% chloramphenicol respectively) into Aniline blue-YM agar as the same way as YM agar with antibiotics.

(3) Procedures

The four kinds of agar media were poured into the disposable petri dishes (100 X 15mm, Falcon), allowed to solidify and held at room temperature overnight before use. The homogenized inocula of the tested organisms were introduced into the individual wells of a sterile Microtiter plate (Dynatech Lab, Alexandria, Virginia) to form a 'master plate'. From this 'master plate', organisms were transferred onto the agar surface of different media in 6 duplicates by use of a multiple inoculator. All the inoculated plates were incubated at 21 C and observed under incandescent and long UV light (366nm) every 12 hours. The results were recorded as growth, no growth, fluorescence and no fluorescence.

III. Performing Physiological Tests of Yeast Identification by Fung's Miniaturized System:

A comparative study of the accuracy and efficiency of Fung's miniaturized system compared with conventional methods was made for stock yeast culture identification. Twenty three physiological tests based on the simplified identification key to food-borne yeast from meat products were involved in this study using both methods.

(1) Fung's Mini System

i. Cultures

Ten yeast cultures listed in Table 13 were used in this study. All the cultures were purified by streaking on the YM agar plate before using as the inoculum. The single colony was picked up with the inoculation loop, then transferred to a sterile test tube with 1 ml sterile distilled water. The test tube was agitated with Vortex to homogenize the inoculum. The 'master plate' was prepared by transferred four drops (ca. 0.2 ml) of the homogenized inocula into the individual wells of the Microtiter. One Microtiter plate accommodates up to 96 cultures. Mass inoculation of yeast from the 'master plate' to solid and liquid media was achieved by use of a sterile multiple inoculator. All the tests were done in duplicate.

ii. Carbohydrate assimilation

The following carbohydrates were used for carbohydrate assimilation tests: galactose, sucrose, maltose, cellobiose, D-ribose, L-rhamnose, erythritol, trehalose, lactose,

Table 13. Yeast Cultures Used in the Comparative Study of Performing 23 Physiological Tests by Fung's Mini System and Conventional Method

Genera	Species	Source	Stock Code
Candida	ablicans	ISU 813-14A	I-8
Candida	parapsilosis	ISU MM-14	I-13
Candida	valida	NRRL Y-936	N-1
Cryptococcus	infirmo-miniatus	NRRL Y-1586	N-18
Cryptococcus	laurentii	NRRL Y-2536	N-19
Cryptococcus	maerans	NRRL Y-5797	N-20
Debaryomyces	hansenii	NRRL Y-7265	D-1
Debaryomyces	marama	NRRL Y-2171	N-4
Pichia	media	NRRL Y-7122	N-9
Trichosporon	cutaneum	NRRL Y-	N-4

Note: ISU = Iowa State University (Ames, IA)

NRRL = Northern Regional research Laboratory (Peoria, IL)

raffinose, soluble starch, D-xylose, L-arabinose, succinic acid, citric acid and inositol. These were selected on the basis of the simplified identification key (Table 9). The stock solutions of individual carbohydrate were made by dissolving 1 gram of carbohydrate in 20 ml distilled water and sterilized with a syringe filter systetm (Acrodisc,0.45 um). The basal agar for the carbohydrate assimilation test contained 2% agar, 0.016% bromcresol purple, 05% ammonium sulfate ($(NH_4)_2SO_4$), 0.1% potassium phosphate monobasic (KH_2PO_4), and 0.05% manganous sulfate ($MgSO_4$). To make 0.5% carbohydrate assimilation test media, 5 ml stock solution of 5% carbohydrate was incorporated into 45 ml sterilized basal medium, which was tempered at a 50C water bath. The media then were poured into the disposable petri dish (Fisher, 150 X 15mm). After hardening, the plates were inoculated with various inocula by the multiple inoculator from the 'master plate'. The inoculated plates were incubated at 21 C. The observations were made every twelve hours for 14 days. Assimilation was considered negative where there was no significant difference between the plate with carbohydrate and without carbohydrate.

iii. Carbohydrate fermentation

There were five carbohydrates used in the fermentation test according to the simplified identification key (Table 9). The stock solutions of 20% glucose, galactose, sucrose, maltose and lactose were made by dissolving 4 grams of

carbohydrate in 20 ml distilled water. The syringe filtration system (Acrodisc, 0.45um) was used to sterile the carbohydrate stock solutions. The basal medium for fermentation test contained 0.45% yeast extract, 0.75% soy peptone, and 0.016% bromcresol purple. To make 2% carbohydrate fermentation liquid media, 2 ml of 20% carbohydrate stock solution was added aseptically to 18 ml of sterilized fermentation basal medium. The Microtiter plates were used as growth chambers and the volume of liquid medium was 0.2 ml per well (four drops of pasteur pipette). After inoculation by the multiple inoculator from the 'master plate', all the wells were overlaid with sterilized mineral oil and then sealed with plate sealers (Dynatech) to trap the gas produced. Incubation was made at 21 C and the observations were made every twelve hours for 14 days. The fermentation was considered positive where the color of the well changed from purple to yellow.

iv. Growth in Vitamin Free Media

Bacto vitamin-free yeast base (Difco) was used for this test. There were two steps in this test, to prevent carrying-over of vitamin from the inocula. Liquid medium was used in the first step to 'exhausted' vitamins in the inocula. The liquid medium was dispensed into the individual wells of the Microtiter (ca. 0.2 ml). The inocula were transferred from the 'master plate'. The inoculated Microtiter plates were incubated at 21 C for 3 days and then from this Microtiter plate, cultures were transferred onto a petri dish plate

containing 2% agar and vitamin-free base with the pin tail of the multiple inoculator. The petri-dish plate was incubated at 21 C for 3 days and read with the aid of the Microtiter reader. The vitamin free test was considered positive where there was a significant growth of the colony.

iv. Growth at 37 C

The yeast inocula were inoculated by multiple inoculator onto YM agar plate from the 'master plate' and incubated at 37 C for 48 hours. The room temperature culture served as the control for viability of the cultures. The growth at 37 C was considered positive where there was a pronounced growth of the colony.

(2) Conventional Methods

i. Carbohydrate Assimilation

Wickerham's liquid medium procedure was used as the comparison of the carbohydrate assimilation tests. The carbohydrates, basal medium and the procedures of preparing stock solution of carbohydrates were same as that in Fung's miniaturized system. However, the concentration of carbohydrates in the medium was 1%. For inoculation, 0.1 ml of yeast suspension was inoculated into the liquid assimilation medium. All the tubes were incubated at 21 C and the observations were made every 24 hours for 14 days.

ii. Carbohydrate Fermentation

The carbohydrates, the basal medium and the procedures of preparing stock solution of carbohydrates for the

fermentation tests are same as that in Fung's miniaturized system. Each test tube was inoculated with 0.1 ml of yeast suspension. After inoculation 1 ml of sterilized mineral oil was added to each tube on the top of the broth. The incubation was made at 21 C and the readings were made every 24 hours for 14 days.

iii. Growth in Vitamin Free Media

The medium and the preparation were same as that in Fung's system. However, 4 ml of medium in each test tube and each test tube was inoculated with 0.1 ml of yeast suspension. The tubes were incubated at 21 C for a week and the yeast was transferred from the first tube into the second tube. The readings were made after incubation at 21 C for another week.

iv. Growth at 37 C

Purified yeast was inoculated by loop onto YM agar slant and incubated at 37C. Observation was made every 24 hours.

IV. Isolation and Identification of Yeasts from Meat Products

Various meat samples were obtained from Meats Laboratory, Kansas State University and local grocery stores. Five grams of meat were put in the sterile Stomacher bag (Seward Medical) with 45 ml of sterile distilled water. The Stomacher bag was homogenized at a Stomacher (Lab Blender 400) for one minute. Homogenized sample solutions were streaked onto antibiotics containing YM agar (100 ppm chlortetracycline and chloramphenicol, respectively) plates. The plates were incubated at 21 C for 3 to 5 days. Single colonies were pickedand transferred to a test tube with 1 ml sterile distilled water. After agitating the test tube with Vortex (Fisher) for 30 second, the homogenized isolates were streaked onto the YM agar plates. The plates were incubated at 21 C for another 3 to 5 days.

The isolates were examined microscopically under 400 times magnification to observe for the shape, size and the budding of the cells.

All the isolates were streaked on 100 ppm Crystal violet containing YM agar plate for the presumptive identification of Candida lipolytica.

V. Presumptive Identification of Known Yeasts Cultures and Fresh Yeast Isolates from Meats Products.

Twenty one known cultures (Table 14) and 41 fresh isolates were identified by using the simplified identification key. twenty three physiological tests using Fung's Mini System as described in part III were performed. The interpretation was made by using MYID23 (Su/Li) and MYID14 (Su/Li) computer programs. The operation system of the computer is MS-DOS and the hardware is Zenth Data Systems.

Microsopic examination of the yeasts was performed to confirm their identities.

Table 14. Yeast Cultures Used in Rapid Presumptive ID by Using Simplified Identification Key

Genera	Species	Source	Stock Code
Candida	albicans	ISU 813-12A	I-7
Candida	albicans	ATCC 10261	C-3
Candida	albicans	ISU 813-14A	I-8
Candida	famata	NRRL Y-1453	C-6
Candida	parapsilosis	NRRL Y-2315	C-14
Candida	parapsilosis	ISU MM-14	I-13
Candida	valida	NRRL Y-936	N-1
Candida	vini	NRRL Y-6658	C-27
Cryptococcus	laurentii	NRRL Y-2536	N-19
Debaryomyces	hansenii	NRRL Y-7265	D-1
Debaryomyces	hansenii	NRRL Y-7425	D-2
Debaryomyces	hansenii	NRRL Y-1454	D-3
Debaryomyces	marama	NRRL Y-2171	N-5
Debaryomyces	marama	NRRL Y-2171	N-24
Pichia	etchellsii	NRRL Y7121	N-8
Pichia	media	NRRL Y-7122	N-9
Pichia	membranaefaciens	NRRL Y-6776	P-2
Pichia	membranaefaciens	NRRL Y-6776	I-20
Saccharomyces	cerevisiae	NRRL Y-2034	S-5
Saccharomyces	cerevisiae	ATCC 4126	I-24
Wickerhamiella	domercqii	NRRL Y-6692	N-14

Note: ATCC = American Type Culture Collection (Rockville, MD).
ISU = Iowa State University (Ames, IA).
NRRL = Northern Regional Research Laboratory (Peoria, IL)

RESULTS AND DISCUSSION

I. The Effect of Some Triarylmethane Dyes on Yeasts Growth

The results of the effects of 20 triarylmethane dyes on the growth of yeast were recorded in Table 15. All the yeasts tested grew on YM agar medium (Medium No. 21, see Table 10) with white colonies except Rhodotorula minutea which had a characteristic pink colony. On Medium 19 (Crystal violet), Medium 6 (Flexo violet 615), and Medium 5 (Flexo violet 600), only Candida lipolytica (ATCC 34088 and ATCC 20362) could form white colony, wherase all other yeasts could not. This result agreed with that of Lin and Fung (1985). On Medium 16 (Brilliant green) and Medium 3 (Basonly blue), no yeast could form colony, which implied that the concentration (1:10,000 dilution) tested was too high for the yeasts to grow. Therefore, less concentrated dye (Brilliant green and Basonly blue) in the agar was needed for the further investigation.

On Medium 17 (Aniline blue), most yeast produced blue or white colonies, but Candida albicans (ATCC 10261) could fluoresce under long wave UV light (366nm) after 24 hours incubation at 21 C. Candida pulcherrima (ISU HB-29) had weak fluorescence after 48 hour incubation at 21 C. Since Candida albicans is one of the most important pathogenic yeast for the human (Rose and Harrison, 1970), further investigation on medium 17 is valuable to develop a rapid presumptive medium for Candida albicans. Goldschmidt et al (1989) investigated a large number of Candida isolates from mouth rinsing from

Table 15. Effect of Some Triarylmethane Dyes on Yeast Growth

	1	2	3	4	5	6	7	8	9	10	11	12
C. lipolytica ATCC34088	G	W	–	W	W	W	W	–	G	G	W	–
C. lipolytica ATCC 20362	G	W	–	W	W	W	W	W	G	G	W	G
C. albicans ATCC 10261	W	W	–	W	–	–	W	–	W	W	W	W
C. catenulata NRRL Y-1508	W	W	–	W	–	–	W	W	W	W	W	W
C. famata NRRL Y-1453	W	–	–	W	–	–	W	–	W	W	W	W
C. gropengiesseriNRRLY-7572	W	–	–	W	–	–	W	–	W	W	W	W
C. krusei ISU HB-2	W	W	–	W	–	–	W	W	W	W	W	W
C. lactis-condensiNRRLY-1515	W	W	–	W	–	–	W	–	W	W	W	W
C. parapsilosis NRRL Y-2315	W	W	–	W	–	–	W	W	W	W	W	W
C. parapsilosis ISU MM-4	W	W	–	W	–	–	W	–	W	W	W	W
C. kefyr NRRL Y-1622	W	W	–	W	–	–	W	–	W	W	W	W
C. pulcherrima ISU HB-29	W	–	–	W	–	–	W	–	W	W	W	W
C. reukarfii NRRL Y-6343	W	–	–	W	–	–	W	–	W	W	W	W
C. sake NRRL Y-1622	W	W	–	W	–	–	W	–	W	W	W	W
C. tropicalis NRRL Y-1552	W	–	–	W	–	–	W	–	W	W	W	W
C. utilis NRRL Y-900	W	–	–	W	–	–	W	W	W	W	W	W
C. vini NRRL Y-6658	W	W	–	B	–	–	W	–	W	W	W	W
C. rugosa NRRL Y-1496	W	W	–	W	–	–	W	–	W	W	W	W
C. steatolytica NRRL Y-7136	W	W	–	W	–	–	–	–	W	W	–	W
H. anomala NRRL Y-366	W	W	–	W	–	–	W	W	W	W	W	W
S. cerevisiae NRRL Y-2034	W	W	–	W	–	–	W	W	W	W	W	W
S. cerevisiae NRRL Y-12647	W	W	–	W	–	–	W	W	W	W	W	W
S. chevalieri NRRL Y-12633	W	W	–	W	–	–	W	W	W	W	W	W
S. dairensis NRRL Y-1353	W	W	–	W	–	–	W	–	W	W	W	W
S. globosus NRRL Y-409	W	W	–	B	–	–	–	W	W	W	–	W
S. rosei NRRL Y-866	W	W	–	W	–	–	W	–	W	W	W	W
S. rouxii ISU FY-4	W	–	–	W	–	–	W	–	W	W	W	W
Z. rouxii NRRL Y-2547	W	W	–	–	–	–	W	–	W	W	W	W
D. hansenii NRRL Y-7268	W	–	–	W	–	–	W	–	W	W	W	W
D. hansenii NRRL Y-7426	W	–	–	W	–	–	W	–	W	W	W	W
D. hansenii NRRL Y-1454	W	–	–	W	–	–	W	–	W	W	W	W
D. subglobosus NRRL Y-6666	W	–	–	W	–	–	W	–	W	W	W	W
R. minuta NRRL Y-1589	R	R	–	R	–	–	R	–	R	R	R	R
P. fermentans NRRL Y-1619	W	W	–	W	–	–	W	–	W	W	W	W
P. membranaefaciensNRRL6776	W	W	–	B	–	–	W	–	W	W	W	W
B. anomalus NRRL Y-1415	W	W	–	W	–	–	W	–	W	W	W	W
B. claussenii NRRL Y-1414	W	W	–	W	–	–	W	–	–	W	W	W
T. beigelii NRRL Y-1490	W	W	–	W	–	–	W	–	–	W	W	W
T. pullulans NRRL Y-1522	W	W	–	W	–	–	W	–	G	G	W	W
Bu. alba NRRL Y-6655	W	W	–	W	–	–	W	–	–	W	W	W

Note: W = white colony, G = green colony, B = blue colony, R = red colony
P = pink colony.

* = fluorescence under long wave UV light

Table 15. (continued)

	13	14	15	16	17	18	19	20	21
C. lipolytica ATCC34088	W	B	P	–	B	R	W	R	W
C. lipolytica ATCC 20362	W	B	W	–	B	R	W	R	W
C. albicans ATCC 10261	W	W	W	–	B*	W	–	W	W
C. catenulata NRRL Y-1508	W	W	W	–	W	R	–	R	W
C. famata NRRL Y-1453	W	W	–	–	B	R	–	R	W
C. gropengiesseriNRRLY-7572	W	W	–	–	B	R	–	W	W
C. krusei ISU HB-2	W	W	P	–	B	R	–	W	W
C. lactis-condensiNRRLY-1515	W	W	W	–	B	R	–	W	W
C. parapsilosis NRRL Y-2315	W	W	W	–	B	R	–	W	W
C. parapsilosis ISU MM-4	W	W	W	–	B	R	–	W	W
C. kefyr NRRL Y-1622	W	W	W	–	B	W	–	W	W
C. pulcherrima ISU HB-29	W	W	W	–	B*	W	–	W	W
C. reukarfii NRRL Y-6343	W	W	W	–	B	W	–	W	W
C. sake NRRL Y-1622	W	W	W	–	B	R	–	W	W
C. tropicalis NRRL Y-1552	W	W	W	–	B	R	–	R	W
C. utilis NRRL Y-900W	W	W	W	–	B	R	–	W	W
C. vini NRRL Y-6658	W	W	–	–	B	W	–	W	W
C. rugosa NRRL Y-1496	W	W	W	–	–	R	–	R	W
C. steatolytica NRRL Y-7136	–	W	W	–	B	–	–	–	W
H. anomala NRRL Y-366	W	W	W	–	B	R	–	R	W
S. cerevisiae NRRL Y-2034	W	W	–	–	B	R	–	R	W
S. cerevisiae NRRL Y-12647	W	W	W	–	B	R	–	R	W
S. chevalieri NRRL Y-12633	W	W	W	–	B	R	–	R	W
S. dairensis NRRL Y-1353	W	W	W	–	–	R	–	R	W
S. globosus NRRL Y-409	–	W	W	–	B	–	–	–	W
S. rosei NRRL Y-866	W	W	W	–	B	R	–	R	W
S. rouxii ISU FY-4	W	W	–	–	B	R	–	R	W
Z. rouxii NRRL Y-2547	W	W	P	–	B	R	–	W	W
D. hansenii NRRL Y-7268	W	W	W	–	B	W	–	W	W
D. hansenii NRRL Y-7426	W	W	W	–	B	W	–	W	W
D. hansenii NRRL Y-1454	W	W	W	–	B	W	–	W	W
D. subglobosus NRRL Y-6666	W	W	W	–	B	W	–	W	W
R. minuta NRRL Y-1589	R	R	R	–	R	R	–	R	R
P. fermentans NRRL Y-1619	W	W	W	–	B	R	–	R	W
P. membranaefaciensNRRL6776	W	W	–	–	B	R	–	R	W
B. anomalus NRRL Y-1415	W	W	–	–	B	R	–	–	W
B. claussenii NRRL Y-1414	–	W	–	–	W	R	–	–	W
T. beigelii NRRL Y-1490	W	W	P	–	B	W	–	W	W
T. pullulans NRRL Y-1522	W	B	W	–	W	W	–	W	W
Bu. alba NRRL Y-6655	W	W	W	–	W	W	–	W	W

Note: W = white colony, G = green colony, B = blue colony, R = red colony
P = pink colony.

* = fluorescence under long wave UV light

patients using Aniline blue containing medium, and found that among 18 species of Candida and related yeasts, only Candida albicans and C. parapsilosis could fluoresce after incubation at 30 C for 24-48 hours. Therefore a simple media has been developed which can be used to isolate and identify C. albicans and related Candida from oral and clinical specimens. Figure 1 illustrated that Candida albicans fluoresced under long wave UV light on Aniline blue containing media. Figure 2 was the negative control under incandescent light.

The data indicated that many dyes presently not used for differential isolation of a particular yeast group could be employed for such purpose. The fact that different strains of the same species (e.g. Candida lipolytica, ATCC 34088 and ATCC 20362; Candida parapsilosis, NRRL Y-2034 and NRRL Y-12647; Saccharomyces cerevisiae, NRRL Y-2034 and NRRL Y-12647; Debaryomyces hansenii, NRRL Y-7268 and NRRL Y-1454) gave exactly the same response to certain dyes also provided evidence that dyes are valuable for isolation of specific organisms from food and environment. These dye-containing media are much more effective in isolating specific genera and species and much simpler to separate yeasts from each other compared with using physiological tests such as carbohydrate assimilation and fermentation tests to separate yeast groups

This study revealed that it was possible to substitute dyes for the carbohydrates in physiological tests for the

characterization of yeasts. Sobczak (1985) held the same opinion and some of his research turned into a commercial yeast identification kit called 'Yeast Star' (Lumac, bv, the Netherlands). The advantages of using dyes are that the dyes are less expensive and have thousands of varieties compared to the carbohydrates. However, there are very few data available for the reaction of yeast against dyes (Sobczak, 1981; Sobczak, 1985; Lin and Fung, 1985).

The present study is a preliminary investigation on the effect of some triarylmethane dyes on the growth of yeasts. Among 183 kinds of the triarylmethane dyes (Color Index 3nd), only a very small number of varieties was tested in this study. Also very few species of yeasts were employed. Therefore, it is necessary to do more investigations on this matter. The mechanism of the inhibitory and differentiation of the triarylmethane dyes on yeasts also should be involved in the further studies.

Figure 1. Candida albicans Grown on Aniline Blue-MY Agar under Long Wave UV Light (366 nm)

1. C. albicans (human origin) on YM agar
2. C. albicans (animal origin) on Aniline blue containing YM agar
3. C. albicans (human origin) on Aniline blue containing YM agar

Figure 2. Candida albicans Grown on Aniline Blue-YM Agar and YM Agar Under Incandescent Light

1. C. albicans (human origin) on YM agar
2. C. albicans (animal origin) on Aniline blue containing YM agar
3. C. albicans (human origin) on Aniline blue containing YM agar

II. The Effect of Aniline Blue and Antibiotics on Yeast and Bacteria

Results of the effect of Aniline blue and antibiotics on yeasts and bacteria are recorded in Table 16. The observations were made after 24 hours incubation at room temperature. No fluorescence was found in all bacteria tested in all four kinds of media. No bacteria could grow on antibiotics containing agar, regardless of the presence of Aniline blue. All Candida albicans fluoresce on both Aniline blue containing agar, regardless of the presence of antibiotics. Saccharomyces cervisiae could not fluoresce on all the agar. Candida lipolytica could not fluoresce on Aniline blue containing agar without antibiotics, but it fluoresced on Aniline blue containing agar with antibiotics. However, the intensity of the fluorescence was much weaker than that of Candida albicans. The reason for Candida lipolytica to exhibit weak fluorescence on Aniline blue containing YM agar with antibiotics was unknown and will need further investigation.

Aniline blue, whose C.I. number is 42755 and C.I. name is Acid blue 22, has been routinely used as the collagen stain to show the collagenous and reticulin fibrils, cartilage, bone, amyloid and nuclei (Mallory, 1900; Mallory, 1938). While in diagnostic clinic laboratories, M-FC medium contains 0.1g/L Aniline blue has been used for color differentiation of faecal coliform (Lin, 1986). Holmes (1984) employed 0.01% Aniline

blue in charcoal-yeast extract medium in conjunction of a long-wave UV light to differentiate five species of Legionella. He found that L. pneumonophila did not absorb the dye, but L. micadei, L. dumoffii, L. bozemanii, and L. gormanii could absorb the dye and gave the colonies various shades of blue color.

It is interesting that different microorganisms absorb Aniline blue in different amount under the given conditions. Further study of the mechanism and screening more microorganisms on the dye may be fruitful for the development of more useful diagnostic media for microbiology.

Table 16. Effect of Aniline Blue and Antibiotics on Yeasts and Bacteria

	Agar Media[1]							
	YM		YM+Anti		YM+Dye		YM+Dye+Anti	
Organisms:	G[2]	F[3]	G	F	G	F	G	F
C. albicans ISU 81312A	+	–	+	–	+	+	+	+
C. albicans ISU 81314A	+	–	+	–	+	+	+	+
C. albicans ATCC10261	+	–	+	–	+	+	+	+
C. lipolyticaATCC34088	+	–	+	–	+	–	+	+
S. cerevisiae Andovin	+	–	+	–	+	–	+	–
S. aureus KSU 326	+	–	–	–	+	–	–	–
L.mesenteroidesATCC10830	+	–	–	–	+	–	–	–
E. coli	+	–	–	–	+	–	–	–
E. agglomerans	+	–	–	–	+	–	–	–
Salmonella spp.	+	–	–	–	+	–	–	–
S. liquifaciens	+	–	–	–	+	–	–	–
S. marceseus	+	–	–	–	+	–	–	–

Note: 1 YM = YM agar
YM+Anti = YM agar plus antibiotics
YM+Dye = YM agar plus Aniline blue
YM+Dy+Anti = YM agar plus Aniline blue and antibiotics
2 G = Growth
3 F = Fluorescence under long wave UV light (366 nm).

III. Comparative Study of Performing 23 Physiological Tests in Yeast Identification by Fung's Mini System and Conventional Method.

Results of 23 physiological tests on 10 yeast species, comparing the Fung's mini system and conventional methods, are recorded in Table 17. Results obtained by Fung's mini system corresponded directly to those obtained by conventional methods indicating the reliability of the Fung's mini system to perform these 23 physiological tests for yeast identification. The incubation period necessary for obtaining definite results for most tests was 3 days for the Fung's mini system and 12 days for the conventional method. For vitamin free test, Fung's mini system needed about one week while conventional method needed at least two weeks due to the multiple steps involved in this procedure. For 37 C growth test, both methods needed one day to obtain expected results.

Figure 3 revealed that there was a difference between incubation time of the two methods to reach 90% agreement with expected results. For Fung's mini system, it took 3 days to obtain 90% agreement with the expected positive response from reference source. However, for conventional methods, it took about 12 days to obtain same level of agreement. After 3 days incubation, the agreements with expected results in Fung's mini system started to decline slightly due to false positive results of some colonies. Mirgration of metabolized

Table 17. Comparison of Fung's Mini System with Conventional Methods of Performing 23 Physiological Tests

	GlF		GaF		SuF		MaF	
	Fung's	Conv	Fung's	Conv	Fung's	Conv	Fung's	Conv
Days	3	12	3	12	3	12	3	12
C. albicans	+	+	−	−	+	+	+	+
C. parapsilosis	+	+	+	+	+	+	+	+
C. valida	+	+	−	−	−	−	−	−
Cry.infirmo-miniatus	−	−	−	−	−	−	−	−
Cry. laurentii	−	−	−	−	−	−	−	−
Cry. maerans	+	+	−	−	+	+	+	+
De. hansenii	+	+	−	−	+	+	−	−
De. marama	+	+	−	−	+	+	−	−
P. media	+	+	−	−	+	+	−	−
Tri. cutaneun	−	−	−	−	−	−	−	−

Note:

(1). All the tests were made as described in the text. The abbreviations for the tests are as same as in table 9.

(2). Fung's = Fung's mini system, Conv = Conventional methods.

(3). The times given are the incubation periods to obtain all the expected positive cultures to response.

(4). + = positive , − = negative.

Table 17. (continued)

	LaF		Gal		Suc		Mal	
	Fung's	Conv	Fung's	Conv	Fung's	Conv	Fung's	Conv
Days	3	12	3	12	3	12	3	12
C. albicans	–	–	+	+	+	+	+	+
C. parapsilosis	–	–	+	+	+	+	+	+
C. valida	–	–	–	–	–	–	–	–
Cry.infirmo-miniatus	–	–	+	+	+	+	+	+
Cry. laurentii	–	–	+	+	+	+	+	+
Cry. maerans	–	–	+	+	+	+	+	+
De. hansenii	–	–	+	+	+	+	+	+
De. marama	–	–	+	+	+	+	+	+
P. media	–	–	–	–	+	+	–	–
Tri. cutaneun	–	–	+	+	+	+	+	+

Table 17. (continued)

	Cel		Rib		Rha		Ery	
	Fung's	Conv	Fung's	Conv	Fung's	Conv	Fung's	Conv
Days	3	12	3	12	3	12	3	12
C. albicans	–	–	–	–	–	–	–	–
C. parapsilosis	–	–	–	–	–	–	–	–
C. valida	–	–	–	–	–	–	–	–
Cry.infirmo-miniatus	+	+	–	–	–	–	–	–
Cry. laurentii	+	+	+	+	+	+	+	+
Cry. maerans	+	+	–	–	–	–	+	+
De. hansenii	+	+	–	–	–	–	+	+
De. marama	+	+	–	–	–	–	+	+
P. media	–	–	–	–	–	–	–	–
Tri. cutaneun	+	+	+	+	+	+	–	–

Table 17. (continued)

	Tre		Lac		Raf		sSt	
	Fung's	Conv	Fung's	Conv	Fung's	Conv	Fung's	Conv
Days	3	12	3	12	3	12	10	15
C. albicans	+	+	+	+	−	−	+	+
C. parapsilosis	+	+	−	−	−	−	−	−
C. valida	−	−	−	−	−	−	−	−
Cry. infirmo-miniatus	+	+	+	+	+	+	+	+
Cry. laurentii	+	+	+	+	+	+	+	+
Cry. maerans	+	+	+	+	+	+	−	−
De. hansenii	−	−	−	−	+	+	+	+
De. marama	+	+	−	−	+	+	−	−
P. media	−	−	−	−	−	−	−	−
Tri. cutaneun	+	+	+	+	+	+	+	+

Table 17. (continued)

	Xyl		Ara		SAd		CAd	
	Fung's	Conv	Fung's	Conv	Fung's	Conv	Fung's	Conv
Days	3	12	3	12	3	12	3	12
C. albicans	+	+	−	−	−	−	+	+
C. parapsilosis	+	+	+	+	−	−	+	+
C. valida	−	−	−	−	−	−	−	−
Cry. infirmo-miniatus	−	−	+	+	+	+	+	+
Cry. laurentii	+	+	+	+	−	−	+	+
Cry. maerans	+	+	+	+	+	+	+	+
De. hansenii	+	+	+	+	+	+	+	+
De. marama	+	+	+	+	+	+	+	+
P. media	−	−	−	−	−	−	+	+
Tri. cutaneun	+	+	+	+	+	+	+	+

Table 17. (continued)

	Ino		Vfr		37C	
	Fung's	Conv	Fung's	Conv	Fung's	Conv
Days	3	12	6*	14**	1	1
C. albicans	–	–	–	–	+	+
C. parapsilosis	–	–	–	–	–	–
C. valida	–	–	–	–	–	–
Cry.infirmo-miniatus	+	+	–	–	–	–
Cry. laurentii	+	+	–	–	–	–
Cry. maerans	+	+	–	–	–	–
De. hansenii	–	–	–	–	+	+
De. marama	–	–	–	–	–	–
P. media	–	–	–	–	+	+
Tri. cutaneun	–	–	–	–	–	–

Note:

* For Vfr (vitamin free) test, the readings of growth of the culture on secondary vitamin free media were taken.

Figure 3. The Relationship of the Agreement with the Expected Results and Incubation Time in Fun g's Mini System and Conventional Method

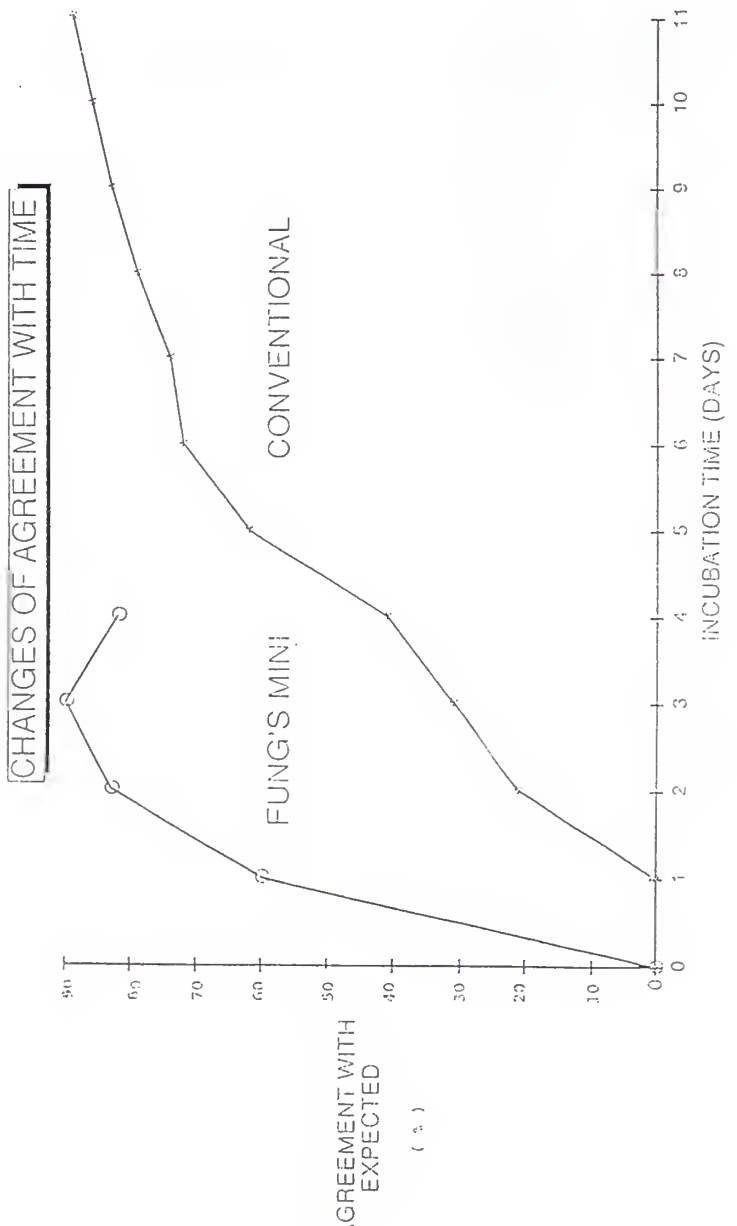
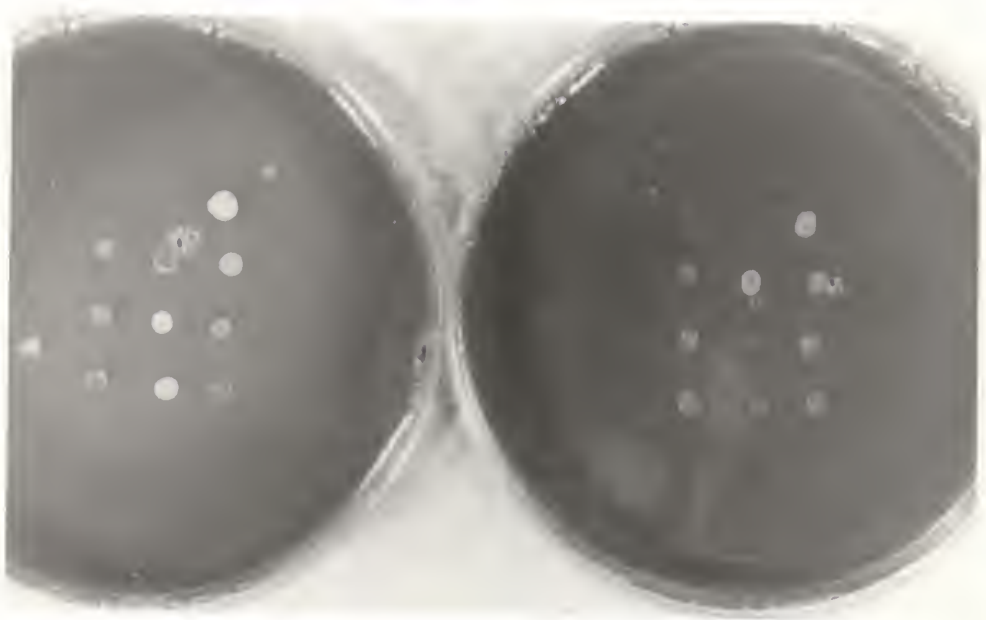


Figure 4. Fasle Positive Caused by Overtime Incubation of Carbohydrate Assimilation Test in Fung's Mini System

* Left plate was 7 days incubation. The middle colony expectedly is a negative response (such as in the right plate)

* Right plate was 2 days incubation



intermediate compounds from adjacent positive colonies stimulated growth of an otherwise negative culture. This could be prevented by reading the results at 3 days and not thereafter. Since the media were added with bromcresol purple as the indicator, it was easy to monitor the progress of the reactions due to interfering metabolites (Figure 4).

The advantages of the Fung's mini system are its saving in time, and materials. For example, only 1.04 mg of carbohydrate was required for each yeast in the carbohydrate assimilation test in Fung's mini system, because 40 mg for each yeast in the conventional method were required. In the carbohydrate fermentation tests, 4 mg of carbohydrate were needed for each yeast in Fung's mini system, whereas 100 mg of carbohydrate was needed for conventional method. The advantages become more obvious when a large number of yeasts are being identified using a large number of carbohydrate compounds as suggested by Wickerham and Burton (1948).

Although incubation times for growth at 37 C test were almost same in both methods, Fung's mini system was more ideal due to its saving space, agar, and short time needed for inoculation and data collection.

IV. The Results of Yeast Isolates from Meat Products

Forty one yeast isolates from meat samples were obtained (Table 18). None of the yeast was Candida lipolytica since none of them can grow on the Crystal violet containing YM agar (C. lipolytica media) developed in this laboratory.

The number of yeast isolates in Table 18 did not provide the quantitive comparison of the yeast flora among these meat samples. The isolates listed in Table 18 were only those purified and further tested.

Table 18. The Results of Yeast Isolates from Various Meat Samples

Meat Samples	Number of Isolates
Chicken (sliced & smoked)	1
Fresh Pork	8
Ground Pork	10
Hot Dog	11
Lunch Bologna	9
Pepperoni (Baby Combo Pizza)	2

V. Rapid Presumptive Identification of Yeasts by Simplified Identification Key for Known Cultures and Fresh Isolates from Meat Products.

Twenty one named yeast cultures and 41 fresh yeast isolates were tested with 23 physiological tests by using Fung's mini system. The results were interpreted by using MYID23 (Su/Li) computer program. However, the output file of interpreted results were disappointing, because almost all the cultures tested were not identifiable.

There are several possible reasons. First, the simplified identification key was made with some subjective assumptions, that is, we simplified the key by using only three symbols (+,-,v) to describe all the physiological attributes. In reality, it was not that clear cut. In the original descriptions (Appendix 1), more than seven symbols were used. Also, the descriptions of the attributes in the simplified identification key were cited from data which were based on typical yeast cultures, while our named cultures might not be typical ones. Therefore, the variation between data from published results and our laboratory test results were not surprising. Since the simplified identification key was made based on the 'fingerprint' of individual yeast, the results of every test for each yeast were extremely critical for the data interpretation. Among twenty three tests, one false response of a test culture compared with the database could

result in a wrong interpretation. In the routine practice, false response are common place for new isolates compared with 'typical' culture collection data.

There are two alternatives to overcome the above problem. One is to collect all the named yeast cultures listed in the key, then test all of them with 23 physiological tests. This approach can make the identification key quite accurate, providing the cultures we use are 'typical' ones. However, to study 84 species of yeast with 2 strains for each species using 23 physiological tests, each test is at least 3 different experimental conditions, and each experimental condition in duplicates, a minimal number of 23184 tests would have to be performed making this approach impractical.

Another approach is to delete some physiological tests from the simplified identification key. This would decrease the accuracy of the key, but still could key-out the unknowns as one of a small number of possibilities. Further examinations, by some supplemental physiological tests or simple morphological tests, would possibly confirm the identity of the unknown.

In this study, a 14 test identification key was proposed. Five fermentation tests (GlF, GaF, SuF, MaF and LaF), three assimilation tests (Lac, sSt and Xyl) and vitamin free test were deleted from the original 23 test identification key. A computer program MYID14 (Su/Li) was facilitated for interpreting results with this modified

identification key. The output files for data interpretation of 21 named yeast cultures and 41 fresh yeast isolates were documented in Appendix 3. Table 19 summarized the results of the data interpretation by using 14 physiological tests of 21 named yeast cultures. Three named cultures, S. cerevisiae(S-5), S. cerevisiae(I-24), and Wickerhamiella domercqii(N-14), were correctly and accurately identified without the need for further experimentation. Fifteen cultures had more than one names given in the output files. Further investigation clarified their identities. Only three cultures, C. famata(C-6), C. parapsilosis(C-14), and De. hansenii(D-2), were not correctly identified by this key. The photomicrographs for yeast cultures identified were in the Appendix 4.

Table 20 summarized the 14 physiological tests for 41 fresh yeast isolates and the presumptive identification by MYID14 (Su/Li). The photomicrographs for 41 isolates are in Appendix 5, and the further presumptive interpretation of the results by comparing the photographs is summarized in Table 21.

Table 19. Data Interpretation and Remarks for Named Yeast Cultures

Code	Named Cultures	Interpretation by MYID14	Remarks
I-3	Candida albicans	C. albicans Saccharomyces cerevisiae	C. albicans can fluoresce on aniline blue MY agar, germ tube test, etc.
I-7	Candida albicans	C. albicans S. cerevisiae	
I-8	Candida albicans	C. albicans S. cerevisiae	
C-6	Candida famata	C. versatilis Sporobolomyces roseus	Wrong identification
C-14	Candida parapsilosis	unknown yeast ???	Wrong identification
I-13	Candida parapsilosis	C. ablicans C. azyma C. parapsilosis C. tropicalis	Germ tube test, and aniline blue test
N-1	Candida valida	C. valida C. vini Pichia membranaefaciens S. cerevisiae	Pichia: liberated ascospores, Saccharomyces: ascospores not liberated
C-27	Candida vini	C. valida C. vini P. membranaefaciens S. cerevisiae	
N-14	Wickerhamiella domercqii	Wickerhamiella domercqii	
N-19	Cryptococcus laurentii	C. curvata C. humicola Cry. laurentii Tri. cutaneum	Cryptococcus spp. usually have carotenoid pigments, mucous colony
D-1	Debaryomyces hansenii	De. hansenii Rhodotorula rubra	Rhodotorula spp. have carotenoid pigments
D-2	De. hansenii	C. famata Rhodotorula glutinis	Wrong identification
D-3	De. hansenii	De. hansenii Rh. rubra	

Table 19 (continued)

Code	Named Cultures	Interpretation by MYID14	Remarks
N-5	De. marama	C. famata C. fenniea C. humicola C. membranaefaciens De. marama De. polymorphus	Debaryomyces spp. have ascospores
N-24	De. marama	C. famata C. fenniea C. humicola C. membranaefaciens De. marama De. polymorphus	
N-8	Pichia etchellsii	C. tropicalis P. etchelsii	Pichia spp. have ascospores
N-9	Pichia media	unknown yeast ???	Wrong identification
P-2	Pichia membranaefaciens	P. membranaefaciens S. cerevisiae S. telluris	Pichia spp. liberated ascospores
I-20	P. membranaefaciens	P. membranaefaciens S. cerevisiae S. telluris	Saccharomyces spp ascospores not liberated
S-5	Saccharomyces cerevisiae	S. cerevisiae	
I-24	S. cerevisiae	S. cerevisiae	

Table 20. Results of 14 Physiological Tests of the Fresh Isolates from Meats and the Presumptive ID by MYID14

Meat samples:	Ga	Su	Ma	Ce	Ri	Rh	Er	Tr	Ra	Ar	Sa	Ca	In	37	The presumptive ID
Ground pork(1)	-	+	-	-	-	-	-	-	-	-	-	-	-	-	S. cerevisiae
(2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P. membranaefaciens
															S. cerevisiae
															S. tulluris
(3)	-	+	-	-	-	-	-	-	-	-	-	-	-	-	S. cerevisiae
(4)	-	+	-	-	-	-	+	-	-	-	-	-	-	-	Unknown
(5)	-	+	-	-	-	-	-	-	-	-	-	-	-	-	S. cerevisiae
(6)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C. valida
															C. vini
															P. membranaefaciens
															S. cerevisiae
(7)	-	+	-	-	-	-	-	+	-	-	-	-	-	-	S. cerevisiae
															T. delbrueckii
(8)	-	+	-	-	-	-	-	+	-	-	+	-	-	+	Unknown
(9)	-	+	-	-	-	-	-	+	-	-	-	-	-	-	S. cerevisiae
															T. delbrueckii
(10)	+	+	-	-	-	-	-	+	-	-	-	-	-	-	S. cerevisiae
															T. delbrueckii
Hot dog (1)	-	-	-	-	-	-	-	+	-	-	-	-	-	-	S. cerevisiae
															T. delbrueckii
(2)	-	-	-	-	-	-	-	+	-	-	-	-	-	-	S. cerevisiae
															T. delbrueckii
(3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C. valida
															C. vini
															P. membranaefaciens
															S. cerevisiae
(4)	-	-	-	-	-	-	-	+	-	-	-	-	-	-	S. cerevisiae
															T. delbrueckii
(5)	-	-	+	-	-	-	-	+	-	-	-	-	-	-	S. cerevisiae
															T. delbrueckii
(6)	-	+	+	-	-	-	-	+	-	-	-	-	-	-	S. cervisiae
															T. delbrueckii
(7)	-	+	+	-	-	-	-	-	-	-	-	-	-	-	S. cerevisiae
(8)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C. vini
															C. valida
															P. membranaefaciens
															S. cerevisiae
(9)	-	+	-	-	-	-	-	+	-	-	-	-	-	-	S. cerevisiae
															T. delbrueckii
(10)	-	+	-	-	-	-	-	+	-	-	-	-	-	-	S. cerevisiae
															T. delbrueckii
(11)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S. cerevisiae
															C. valida
															C. vini
															P. membranaefaciens

Table 20. (continued)

Meat samples:	Ga	Su	Ma	Ce	Ri	Rh	Er	Tr	Ra	Ar	Sa	Ca	In	37	The presumptive ID
Bonlonga (1)	+	+	+	+	-	-	-	+	+	+	+	-	-	-	C. famata R. glutinis
(2)	+	+	+	-	-	-	-	+	+	+	+	-	-	-	Unknown
(3)	+	+	+	+	-	-	-	+	+	-	-	-	-	-	C. versatilis Sp.paraoseus Sp.roseus
(4)	+	+	+	+	-	-	-	+	+	+	-	-	-	-	C. versatilis Sp.roseus
(5)	+	+	+	-	-	-	-	-	+	-	-	-	-	-	S. cerevisiae
(6)	+	+	+	+	-	-	-	+	+	+	-	-	-	-	C. versatilis Sp.roseus
(7)	+	+	+	+	-	-	-	+	+	+	-	-	-	-	C. versatilis Sp. roseus
(8)	+	+	+	+	-	-	-	+	+	+	-	-	-	-	C. versatilis Sp.roseus
(9)	+	+	+	+	-	-	-	+	+	+	-	-	-	-	C. versatilis Sp.roseus
Fresh Pork(1)	+	+	+	+	-	-	+	+	+	+	+	-	-	-	C. famata
(2)	+	+	+	+	-	-	+	+	+	+	+	-	-	-	C. famata
(3)	+	+	+	+	-	-	+	+	+	+	+	-	-	-	C. famata
(4)	+	+	+	+	-	-	+	+	+	+	+	-	-	-	C. famata
(5)	+	+	+	+	-	-	+	+	+	+	+	-	-	-	C. famata
(6)	+	+	+	+	-	-	+	+	+	+	+	-	-	-	C. famata
(7)	+	+	+	+	-	-	+	+	+	+	+	-	-	-	C. famata
(8)	+	+	+	+	-	-	-	+	+	-	-	-	+	-	Unknown
Pepperoni (1)	+	+	+	-	-	-	-	+	-	-	-	-	-	-	C. versatilis S. cerevisiae T. delbrusckii
(2)	-	+	-	-	-	-	-	+	-	-	-	-	-	-	S. cerevisiae T. delbrueckii
Chicken (1)	-	+	+	+	+	+	+	+	+	+	-	-	-	+	Unknown

* Notations:

Ga = Galactose assimilation
Su = Sucroes assimilation
Ma = Maltose assimilation
Ce = Cellobiose assimilation
Ri = D-Ribose assimilation
Rh = L-Rhamnose assimilation
Er = Erythritol assimilation
Tr = Trehalose assimialtion
Ra = Paffinose assimilation
Ar = L-Arabinose assimilation
Sa = Succinic acid assimilation
Ca = Citric acid assimilation
In = Inositol assimilation
37 = Growth at 37C

Table 21. Further Presumptive ID of Yeast Isolates from Meat

Meat	Picture #	Presumptive ID by MYIDI4	Further presumptive ID by morphological observation
Ground pork	A 1	S. cerevisiae	not S. cerevisiae
	A 2	P. membranaefaciens S. cerevisiae S. tulluris	possibly S. cerevisiae
	A 3	S. cerevisiae	not correct, it is bacterium by morphology
	A 4	unknown	
	A 5	S. cerevisiae	S. cerevisiae
	A 6	C. valida C. vini P. membranaefaciens S. cerevisiae	C. valida similar to E 7
	A 7	S. cerevisiae T. delbrueckii	T. delbrueckii differs from A 5
	A 8	unknown	
	A 9	S. cerevisiae T. delbrueckii	S. cerevisiae similar to A 5
	A 10	S. cerevisiae T. delbrueckii	S. cerevisiae
Hot dog	A 11	S. cerevisiae T. delbrueckii	S. cerevisiae
	A 12	S. cerevisiae T. delbrueckii	S. cerevisiae
	B 1	C. valida C. vini P. membranaefaciens S. cerevisiae	S. cerevisiae
	B 2	S. cerevisiae T. delbrueckii	S. cerevisiae
	B 3	S. cerevisiae T. delbrueckii	S. cerevisiae
	B 4	S. cerevisiae T. delbrueckii	S. cerevisiae

CONCLUSIONS

1. Triarylmethane dyes are very promising for developing new media for yeast isolation and presumptive identification.

2. On Aniline blue containing YM agar (1:10,000), only Candida albicans could fluoresce under long UV (366 nm) light after 24 hours incubation at 21 C. C. pulcherrima fluoresced faintly after 48 hour of incubation.

3. On Crystal violet, Flexo violet 615, and Flexo violet 600 containing YM agar (1:10,000), only Candida lipolytica formed white colony after 24 hours incubation at 21 C.

4. No bacteria could grow on antibiotics containing YM agar. No bacteria could fluoresce on the Aniline blue containing YM agar with or without antibiotics.

5. On Aniline blue plus antibiotics containing YM agar, Candida albicans fluoresced brightly after 24 hour incubation at 21 C. Candida lipolytica fluoresced weakly after 48 hour incubation at 21 C.

6. The results of 23 physiological tests by Fung's mini system corresponded directly to those by conventional methods, but the Fung's mini system can save labor, time, material, and space.

7. For most physiological tests, Fung's mini system only needed 2 to 3 days to obtain expected positive results, while conventional methods needed about 10 to 15 days.

8. Two computer programs for presumptive identification of food-borne yeast were developed with database for 84

species of yeasts. Program MYID23 (Su/Li) could interpret results of 23 physiological tests and MYID14 (Su/Li) could interpret results of 14 physiological tests.

9. The MYID14 (Su/Li) computer program was found to be practical for presumptive identification of food-borne yeast in meat products.

10. Eighteen out of 21 named yeast cultures were correctly identified by using MYID14 (Su/Li) computer program.

11. Forty one fresh yeast isolates from various meat products were presumptively identified by MYID14 (Su/Li) computer program. The results shown that different meat products had various yeast flora. Yeasts identified included Candida valida, C. famata, C. versatilis, Saccharomyces cerevisiae and Torulopsis delbrueckii.

12. Photomicrographs (phase contrast) for 21 named yeast cultures and 41 fresh yeast isolates were presented for morphological confirmation of identities of the food-borne yeasts.

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APPENDIX

I. Physiological Attributes of 84 Food-borne Yeast in Meat Products:

(All the data were complied from ' The Yeasts, a taxonomic study' third revised and enlarged edition, edited by N.J.W. Kreger-van Rij , Elsevier 1984)

	Glu.F	Gal.F	Suc.F	Mal.F	Lac.F	Raf.F	Fre.F
Bullera alba	-	-	-	-	-		-
B. isugae	-	-	-	-	-		-
Candida albicans	+	v	-(+)	+	-		
C. azyma							
C. blankii	- or +s	-	-	-	-		-
C. buffonii							
C. catenulata	v	-	-	-	-		
C. ciferrii							
C. curvata							
C. didensiae	+ or s	- or +s	- or +w	- or +w	-		
C. diversa	+	-	-	-	-		
C. etchellsii	+	-	-	+	-		
C. famata	v	-	v	-	-	v	v
C. fennica	+	+	-	+	- or +s	- or +s	
C. foliorum							
C. glabrata	+	-	-	-	-	+ or w	
C. glaebosa							
C. gropengiesseri	+	-	-	-	-		
C. humicola							
C. inconspicua							
C. intermedia	+	+ or s	-	v	-	v	v
C. ingens							
Candida insectamans	+w or s	-	-	-	-		
C. lambica	+	-	-	-	-		
C. membranaefaciens	+	v	-	-	-	v	v
C. norvegica	v	-	-	-	-		
C. parapsilosis	+	v	- or +w	- or +w	-		
C. pinus	+	+	-	+w or s	-	-	
C. rugosa							
C. sake	+	+s or w	v	v	-		
C. silvae	+w or -	-	-	-	-		
C. silbvatica							
C. tropicalis	+	+	v	+	-		+ or s
C. valida	+s	-	-	-	-		
C. vanderwaltii							
C. versatilis	+	+	v	+	+w or -	-	+
C. vini							
C. zewlanoides	- or -w	-	-	-	-		+s or -
Cryptococcus albidus							
Cry. hungaricus							
Cry. laurentii							

	Gal	L-Sor	Suc	Mal	Cello	D-Ara	D-Rib	L-Rha
Bullera alba	+		+	+	+		+ or w	+
B. tsugae	+		+	+	+		+w	-
Candida albicans	+	v	+(-)	+	-	- or +s	- or +s	-
C. azyma	+	+	+	+	-	v	-	-
C. blankii	-	-	-	-	+	-	-	-
C. buffonii	-	+s	-	+	+	-	-	-
C. catenulata	+	-	-	+	-	-	-	-
C. ciferrii	+		+	+	+		+	v
C. curvata	+	v	+	+ or s	+	-	+	+ or w
C. cidensiae	+	-	+	+	+	-or =s	+ or s	-
C. diversa	-	-	-	-	-	-	-	-
C. etchellsii	+	v	-	+	-	-	v	-
C. famata	+	v	+	+	+	v	v	v
C. fennica	+	+s or -	+	+	v	- or +s	+ or s	-
C. foliorum	-	-	-	-	+w,s	v	v	-
C. glabrata	-	-	-	-	-	-	-	-
C. glucosa	+	+	+	+	+	v	-	-
C. gropengiesseri	+	-	+	-	+	-	-	-
C. humicola	v	v	+	+	v	v	+	v
C. inconspicua	-	-	-	-	-	-	-	-
C. intermedia	+	+	+	+	+	-	- or +s	v
C. lugens	+	v	-	-	-	-	-	-
Candida insectamans	-	-	-	+	+	-	+or s	-
C. lambica	-	-	-	-	-	-	-	-
C. membranaefaciens	+	+	+	+	+	-	+	-
C. norvegica	-	-	-	-	+	-	-	v
C. parasilosis	+	v	+	+	-	-	v	-
C. pinus	+	+	+	+	+	v	+ or s	-s or vw
C. rugosa	+	v	-	-	-	v	-	-
C. sake	+	+	+	+	v	-	- or +s	-
C. silvae	-	-	-	-	-	v	-	-
C. silvanica	-	-	-	-	-	-	-	-
C. tropicalis	+	v	v	+	v	-	v	-
C. valida	-	-	-	-	-	-	-	-
C. vanderwaltii	+	+	+s or -	-	-	+s	+s	-
C. versatilis	+	-	v	+	v	-	v	-
C. vini	-	-	-	-	-	-	-	-
C. zeylanoides	v	+ or s	-	-	v	-	-	-
Cryptococcus albidus	v		+	+ or s	+		v	v
Cry. hungaricus	+		+	+	+		+ or w	+ or w
Cry. laurentii	+		+	+	+		+	+(w)

	Gal	L-Sor	Suc	Mal	Cello	D-Ara	D-Rib	L-Rha
Bullera alba	+		+	+	+		+ or w	+
B. tsugae	+		+	+	+		+w	-
Candida albicans	+	v	+(-)	+	-	- or +s	- or +s	-
C. azyma	+	+	+	+	-	v	-	-
C. blankii	-	-	-	-	+	-	-	-
C. buffonii	-	+s	-	+	+	-	-	-
C. catenulata	+	-	-	+	-	-	-	-
C. citerrii	+		+	+	+		+	v
C. curvata	+	v	+	+ or w	+	-	+	+ or w
C. diddensiae	+	-	+	+	+	-or =s	+ or s	-
C. diversa	-	-	-	-	-	-	-	-
C. eichellsii	+	v	-	+	-	-	v	-
C. famata	+	v	+	+	+	v	v	v
C. fennica	+	+s or -	+	+	v	- or +s	+ or s	-
C. foliorum	-	-	-	-	+w,s	v	v	-
C. glabrata	-	-	-	-	-	-	-	-
C. gliebosa	+	+	+	+	+	v	-	-
C. gropengiesseri	+	-	+	-	+	-	-	-
C. guilliermondii	v	v	+	+	v	v	+	v
C. incospicua	-	-	-	-	-	-	-	-
C. intermedia	+	+	+	+	+	-	- or -s	v
C. lipolytica	+	v	-	-	-	-	-	-
Candida insectamans	-	-	-	+	+	-	+or s	-
C. lambica	-	-	-	-	-	-	-	-
C. membranaefaciens	+	+	+	+	+	-	+	-
C. norvegica	-	-	-	-	+	-	-	v
C. parapsilosis	+	v	+	+	-	-	v	-
C. pinus	+	+	+	+	+	v	+ or s	+s or vw
C. rugosa	+	v	-	-	-	v	-	-
C. sake	+	+	+	+	v	-	- or +s	-
C. silvae	-	-	-	-	-	v	-	-
C. silvanica	-	-	-	-	-	-	-	-
C. tropicalis	+	v	v	+	v	-	v	-
C. valida	-	-	-	-	-	-	-	-
C. vanderwaltii	+	+	+s or -	-	-	+s	+s	-
C. versatilis	+	-	v	+	v	-	v	-
C. vini	-	-	-	-	-	-	-	-
C. zeylanoides	v	+ or s	-	-	v	-	-	-
Cryptococcus albidus	v		+	+ or w	+		v	v
Cry. hungaricus	+		+	+	+		+ or w	+ or w
Cry. laurentii	+		+	+	+		+	+(w)

	Gly	Ery	Rib	Galac	D-Mam	Tre	Lac	Meli
Bullera alba	v	+(-)		+		+	+	
B. tsugae		-	+w			+	+	
Candida albicans	v	-	- or +s	-	+	+	+	-
C. azyma	+ or s	-	v	v	+ or s	+	-	-
C. blankii	+	-	-	-	-	-	-	-
C. boidinii	+s	-	+	-	+	+	-	-
C. catenulata	+	-	-	-	+	-	-	-
C. chiferrii		+	+		+	+	-	
C. curvata	+	+ or w	+ or w	-	v	+	+	-
C. oidensiae	+	+	+	-	+	+	-	-
C. diversa	-	-	+	-	+	-	-	-
C. etchellsii	+	-	-	-	v	-	-	-
C. famata	+	v	+	v	+	+	v	v
C. fennica	+ or s	+	+	-	+	+	v	v
C. foliorum	+s	-	v	-	+	+	-	-
C. glabrata	v	-	-	-	-	+	-	-
C. glaebosa	+	-	+	-	+	+	+	+
C. gropengiesseri	+	-	-	-	+	-	-	-
C. guilliermondii	v	-	v	v	+	+	v	-
C. incospicua	+	-	-	-	-	-	-	-
C. intermedia	-	-	- or +s	-	+	-	+	-
C. krusei	+	-	-	-	-	-	-	-
Candida insectamans	- or +s	-	+	-	+	+	-	-
C. lambica	+	-	-	-	-	-	-	-
C. membranaefaciens	+	-	+	-	+	+	-	+
C. norvegica	+	-	-	-	+	-	-	-
C. parapsilosis	+	-	+ or s	-	+	-	-	-
C. pelliculosa	v	-	+ or s	v	+	+	-	-
C. rugosa	v	-	v	-	+	-	-	-
C. sake	+ or s	-	v	-	+	+	-	-
C. silvae	+	-	+ or s	-	+	-	-	-
C. silvanorum	+	-	+	-	+	+	-	-
C. tropicalis	v	-	v	-	+	+	-	-
C. validn	+	-	-	-	-	-	-	-
C. vanderwaltii	+	-	+	- or +s	+	+	-	-
C. versatilis	+	-	-	-	v	+	v	v
C. vini	v	-	v	-	+	-	-	-
C. zeylanoides	+	-	v	-	+	+	-	-
Cryptococcus albidus		-	v		+(w)	+ or w	v	
Cry. hungaricus		- (+)	v		+	+ or w	v	
Cry. laurentii		-(-)	+	-	v	+	+	

	Raf	Mel	s.Sta	D-Xyl	L-Ara	D-Ara	D-Rib	L-Sha
Bullera alba	+		+ or w	+	+		v	v
B. tsugae	-		-	+w	-		v	-
Candida albicans	-	v	+	+	v		+	-
C. azyma	-	-	-	v	v		-	-
C. blankii	-	-	-	-	-		-	v
C. buffonii	-	-	+	+s	+s		-	-
C. catenulata	-	-	+	+	-			-
C. ciferrii	+		+	+	+		v	-
C. curvata	+	v	+	+	v		v	-
C. didensiae	-	+	-	+	+		-	-
C. diversa	-	-	-	v	-		+	-
C. etchellsii	-	-	-	-	-		+	v
C. famata	+	+	v	+	+		+	-
C. fennica	+ or s	v	+ or s	+s	v		-	-
C. foliorum	-	-	-	+ or s	v		+ or s	-
C. glabrata	-	-	-	-	-		-	-
C. glaebosa	+	v	+w	+	+w,s		-	-
C. gropengiesseri	+	-	-	-	-		+or s(-)	v
C. humicola	v	v	v	+	v		v	v
C. inconspicua	-	-	-	-	-		v	v
C. intermedia	+	-	+	+	v		v	-
C. ingens	-	-	-	-	-		v	v
Candida insectamans	-	- or +s	+	+	-			
C. lambica	-	-	-	+	-			
C. membranaefaciens	+	-	-	+	+			
C. norvegica	-	-	-	+	v			
C. parapsilosis	-	-	-	+	+			
C. pinus	+	-	+	+ or s	- or +s			
C. rugosa	-	-	-	v	v			
C. sake	-	v	-	+	-			
C. silvae	-	-	-	-	-			
C. silbvatica	-	-	-	-	-			
C. tropicalis	-	v	+	+	+w or -			
C. validn	-		-	-	-			
C. vanderwaltii	-	-	-	+	+			
C. versatilis	v	-	-	-	v			
C. vini	-	-	-	-	-			
C. zewlanoides	-	-	-	-	-			
Cryptococcus albidus	+w(+)		v	+ or w	+ or w			
Cry. hungaricus	+ or w		+w	+	+			
Cry. laurentii	+		+ or w	+(w)	+			

	Ery	Rib	Galac	D-Man	D-Gluci	D-Gluci	Sal	Lac Acid
Bullera alba	v	+		+				+
B. tsugae	+	+		+				
Candida albicans	+	+		+		+(-)	-	v
C. azyma	-	-		+ s		+ or s	-	-
C. blankii	-	-		-		-	+	-
C. buffonii	-	-		-		+	+s	-
C. catenulata	-	v		-		+	-	+
C. ciferrii	-	+		-				
C. curvata	-	+		+		v	+	v
C. didensiae	-	+		-		+	+	-
C. diversa	-	-		-		+	-	-
C. etchellsii	-	+		+		+	v	v
C. famata	+	+		+		+	+	+
C. fennica	-	-		-		+	+ or s	- or +s
C. foliorum	+	-		-		+	-	+s
C. glabrata	-	-		-		-	-	-
C. glaebosa	-	-		-		+	+	+
C. gropengiesseri	-	-(+)		-		+	+	-
C. humicola	-	v		v		v	+	v
C. inconspicua	-	v		-(-)		-	-	+
C. intermedia	v	-(-)		-(-)		+	+	-
C. ingens	-	-(-)		v		-	-	v
Candida insectamens						+	+	-
C. lambica						-	-	+
C. membranaefaciens						+	+	v
C. norvegica						+	+	+
C. parapsilosis						+	-	v
C. pinus						+	+	-
C. rugosa						+	-	+
C. suke						+	+ or s	v
C. silvae						+	-	v
C. silvatica						+	-	+
C. tropicalis						+	v	v
C. valida						-	-	v
C. vanderwaltii						+	-	-
C. versatilis						+	v	-
C. vini						+	-	v
C. zewlanoides						+	v	-
Cryptococcus albidus								
Cry. hungaricus								
Cry. laurentii								

	Suc Acid	Cit Acid	Ino	S.Arbu	Nitra	Vit.free 37 C		Urease
Bullera alba	+ or w	+	+s	-	-	-	-	
B. tsugae	+w	+w	-		+	+w	-	
Candida albicans	v	v	-	-	-	-	+	
C. azyma	+	-	-	-	-	-	+	
C. blankii	+w	+w	-	+	+	+	+	
C. buffonii	-	- or +s	-	+	+	-	-	
C. catenulata	v	v	-	-	-	+	v	-
C. chiferrii	+	+ or w	+	+	-	-	+	
C. curvata	v	v	+ or w	+	-	+w	v	+
C. didensiae	+	+	-	+	-	-	+	
C. diversa	+	+	-	-	-	-	v	
C. etchellsii	v	-	-	-	+	v	-	
C. famata	+	v	-	+	-	v	v	
C. fennica	+	+ or s	-	+	-	+ or s	v	-
C. foliorum	+s	+s	-	-	+	+s	+	+
C. glabrata	-	-	-	-	-	-	+	
C. glaebosa	+	+	-	+	-	-	-	
C. gropengiesseri	-	-	-	+	-	+w	-	
C. humicola	v	v	v	+	-	v	-	+
C. inconspicua	+ or w	-	-	-		-	+	
C. intermedia	v	v	-	-	-	-	- or +w	-
C. ingens	v	-	-	-		+	v	+
Candida insectamans	+s	+	-	-	-			
C. lambica	+	+	-	-	-			
C. membranaefaciens	v	v	-	v	-			
C. norvegica	v	v	-	+	+			
C. parasilosis	v	v	-	-	-			
C. pinus	+	+	-	+	-			
C. rugosa	+	v	-	-	-			
C. sake	+	v	-	+ or s	-			
C. silvae	+	v	-	-	-			
C. silvatica	+	-	-	-	-			
C. tropicalis	+	v	-	v	-			
C. valida	v	-	-	-	-			
C. vanderwaltii	+	+	-	-	+			
C. versatilis	-	-	-	v	+			
C. vini	v	-	-	-	-			
C. zewlanoides	+	+	-	+	-			
Cryptococcus albidus	+ or w	+ or w	+		+			
Cry. hungaricus	+w	+w	+w		-			
Cry. laurentii	v	+ or w	+(w)		-			

	Glu.F	Gal.F	Suc.F	Mal.F	Lac.F	Raf.F	Tre.F
Cry. macerans							
Cry. skinneri							
Cry. uniguttulintus							
Debaryomyces castellii	+	-	+	+w or s	-	+	
Debaryomyces hansenii	+	-	-	-	-		
De. marama	-	-	-	-	-		
De. polymorphus	+	+	+	+	-	+ or w	
Filobasidium uniguttulatum	-	-	-	-			
Hansenula californica	+	-	-	-	-	-	
Issatchenkia orientalis	+	-	-	-	-	-	-
Leucosporidium scottii	-	-	-	-			
Metschnikowia polcherrima	+	-	-	-	-	-	
Pichia carsonii	-	-	-	-			
P. etchellsii	+	-	-	-	-	-	-
P. fermentans	+	-	-	-	-	-	-
P. guilliermondii	+	v	+	-	-	+	+
P. haplophiols	-	-	-	-			
P. humboldii	-	-	-	-			
P. media	-	-	-	-			
P. membranaefaciens	-	-	-	-	-	-	-
P. rhodanensis	+	-	-	-	-	-	-
Rhodosporidium infirmo-miniatum	-	-	-	-			
Rhodotorula glutinis	-	-	-	-			
R. graminis	-	-	-	-			
R. minuta	-	-	-	-			
R. rubra	-	-	-	-			
Saccharomyces cerevisiae	+	v	v	v	-		
S. dairensis	+	+	-	-	-		
S. exiguus	+	+	+	-	-		
S. telluris	+	-	-	-	-		
Saccharomycopsis lipolytica	-	-	-	-	-		
Sporidiobolus pararoseus	-	-	-	-	-		
Sporobolomyces salso-ruberseeus	-	-	-	-	-		
S. puniceus	-	-	-	-	-		
S. roseus	-	-	-	-	-		
Stephanoascus ciferrii	-	-	-	-	-		
Torulaspora delbrueckii	+	v	v	v	-		
T. globosa	+	-	+	-	-		
Trichosporon cutaneum	-	-	-	-	-		
Tri. pullulans	+	-	-	-	-		
Wickerhamiella domergii	-	-	-	-	-		
Zygosaccharomyces rouxii	+	-	+	v	-		

	Gal	L-Sor	Suc	Mal	Cello	D-Ara	D-Rib	L-Rha
Cry. macrans	+ or w		+	+ or w	-		+	-(+)
cry. skinneri	+		-	-	+		+	+
Cry. uniguttulatus	v		+	+	-		- or +w	+or w
Debaryomyces castellii	+		+	+	+		-	+
Debaryomyces hansenii	+		+	+	+			
De. marama	+		+	+	+			
De. polymorphus	+		+	+	+			
Filobasidium uniguttulatum	- or +w		+	+	-			
Hansenula californica	-		+	v	+			
Issatchenkia orientalis	-		-	-	-			
Leucosporidium scottii	v		+	+	+			
Metschnikowia pulcherrima	+		+	+	+			
Pichia carsonii	+		+	+	v			
P. etchellsii	+		+	+	+			
P. fermentans	-		-	-	-			
P. guilliermondii	+		+	+	+			
P. haplophiola	+		-	-	-			
P. humboldii	+		-	-	-			
P. media	+		-	+	+			
P. membranaefaciens	-		-	-	-			
P. rhodanensis	-		+	+	+			
Rhodosporidium infirmo-miniatum	+		+	+	+			
Rhodotorula glutinis	+		+	+	+			
R. graminis	+		+	-(+)	v			
R. minuta	v		+(-)	v	v			
R. rubra	v		+	v	v			
Saccharomyces cerevisiae	v		v	v	-		-	-
S. dairensis	+		-	-	-		- or +s	-
S. exiguus	+		+	-	-		-	-
S. telluris	-		-	-	-		-	-
Saccharomycopsis lipolytica	-(+)		-	-	-		-(+)	-
Sporidiobolus pararoseus	+ or s		+	+	+		v	-(-)
Sporobolomyces albo-rubescens	+		+	+	-		-	-
S. puniceus	-		+	+s	+		-s	-
S. roseus	v		+	+	+(-)		v	-
Stephanoascus ciferrii	+		+	+	+		-	v
Torulaspora delbrueckii	v		v	v	-		-	-
T. globosa	-		+	-	-		-	-
Trichosporon cutaneum	+(-)		+(-)	+(-)	+(-)		+(-)	v
Tri. pullulans	+		+	+	+		v	+ or w
Wickerhamiella domergii	v		+w or -	-	-		+	-
Zygosaccharomyces rouxii	v		v	v	-		+s	-

	Gly	Ery	Rib	Galac	D-Man	Trc	Lac	Melt
Cry. macrans		+	+ s		+			
cry. skinneri		- or +w	-		+			
Cry. uniguttulatus		-	s		+			
Debaryomyces castellii		-	-		+			
Debaryomyces hanseni i						+	v	
Dc. marama						+	v	
Dc. polymorphus						+	v	
Filobasidium uniguttulatum						+s	-	
Hansenula californica						v	-	
Issatchenkia orientalis						-	-	
Leucosporidium scottii						+	v	
Metschnikowia pulcherrima						+	-	
Pichia carsonii						+	-	
P. etchellsii						+	-	
P. fermentans						-	-	
P. guilliermondii						+	-	
P. haplophiola						-	-	
P. humboldii						-	-	
P. media						+	-	
P. membranaefaciens						-	-	
P. rhodanensis						+	-	
Rhodosporidium infirmo-miniatum						+	+s or w	
Rhodotorula glutinis						+	-	
R. graminis						+	-	
R. minuta						+	v	
R. rubra						+	-	
Saccharomyces cerevisiae		-	-		v	v	-	
S. dairensis		-	-		-	v	-	
S. exiguus		-	-		-	+	-	
S. telluris		-	-		-	-	-	
Saccharomycopsis lipolytica		+	-(+)		+	-	-	
Sporidiobolus pararoseus		-	v		+	+	-	
Sporobolomyces albo-rubescens		-	-		-	+	-	
S. puniceus		-	-	+	-	+s	-	
S. roseus		-	v		+	+	-	
Stephanoascus ciferrii		+	+		+	+	-	
Torulaspora delbrueckii		-	-(+s)		+(-)	+	-	
T. globosa		-	-	v	v	- or +s	-	
Trichosporon cutaneum		v	v		v	v	+	
Tr. pullulans		+	v		+	+	+ Or w	
Wickerhamiella domergii		-	-		+	-	-	
Zygosaccharomyces rouxii		-	v		v	v	-	

	Raf	Mel	s.Star	D-Xyl	L-Ara	D-Ara	D-Rib	L-Rha
Cry. macrans	+ or w		+s v	+	+			
cry. skinneri	-		-	+	+			
Cry. unigutulatus	+ or w		+w	+	+			
Debaryomyces castellii	+		+	+	+			
Debaryomyces hansenii	+		+	+	+		v	v
De. marama	+		+	+	+		v	-
De. polymorphus	+		+	+	v		+	-
Filobasidium uniguttulatum	v		+w or s	+	+ or s		-	-
Hansenula californica	-		-	+	-		-	v
Issatchenkia orientalis	-		-	-	-		-	-
Leucosporidium scottii	+		-	+	v			+
Metschnikowia pulcherrima	-		-	+	-		v	-
Pichia carsonii	v		+	+	v		v	-
P. etchellsii	-		-	+	+ or w		-	-
P. fermentans	-		-	+	-		+	-
P. guilliermondii	+		-	+	+		+	v
P. haplophiola	-		-	+	+		+	-
P. humboldii	-		-	-	-		-	-
P. media	-		- or +w	+	+		+ or s	-
P. membranaefaciens	-		-	v	-		-	-
P. rhodanensis	-		-	+	-		-	+
Rhodosporidium infirmo-miniatum	+		+	+	+		+or s(-)	v
Rhodotorula glutinis	-		v	+	v		v	v
R. graminis	+		-	+(w)	v		v	v
R. minuta	-(+w)		-	+(-)	+(-)		v	-
R. rubra	+		-	+	v		v	v
Saccharomyces cerevisiae	v		v	-	-		-	-
S. darrensis	-		-	-	-		- or +s	-
S. exiguus	+ or s		-	-	-		-	-
S. telluris	-	-	-	-	-		-	-
Saccharomycopsis lipolytica	-		-	-	-		-(+)	-
Sporidiobolus pararoseus	+		v	v	-		v	-(+)
Sporobolomyces albo-rubescens	+		-	+	+		+	-
S. puniceus	+		-	-	+		+s	-
S. roseus	+		+	v	v		v	-
Stephanoascus ciferrii	+		v	+	+		+	v
Torulaspora delbrueckii	v		-	- or +s	-		-	-
T. globosa	-		-	-	-		-	-
Trichosporon cutaneum	v		-(-)	+	+(-)		+(-)	v
Tri. pullulans	+		+	v	+(-)		v	+ or w
Wickerhamiella domergii	-		-	-	-		+	-
Zygosaccharomyces rouxii	-		-	-	-		+s	-

	Suc acid	Cit Acid	Ino	S.Arbu	Nitra	Vit.free 37 C	Urease
Cry. macrans	+w	+w	+		+		
Cry. skinneri	+w	+w	+		-		
Cry. uniguttulatus	+ or w	v	+		-		
Debaryomyces castellii	+	+	-	+	-		
Debaryomyces hanseni	+	v	-	+	-	- v	
De. marama	+	+	-	+	-	- -	
De. polymorphus	+	+	-	+	-	- v	
Filobasidium uniguttulatum	-	-	+		-	- -	
Hansenula californica	+	v	-		+	- -	
Issatchenkia orientalis	+	-	-		-	+ +	
Leucosporidium scottii	-	-	-	+	+	+ -	+
Metschnikowia pulcherrima	+	v	-		-	- v	
Pichia carsonii	+	+	-		-	- - or +w	
P. etchellsii	+	+	-		-	- +	
P. fermentans	+	+	-		-	- +	
P. guilliermondii	+	v	-		-	- +	
P. haplophila	-	-	-		-	- -	
P. humboldtii	+	-	-		-	- v	
P. media	+	+	-		-	- -	
P. membranaefaciens	v	v	-		-	v v	
P. rhodanensis	+	+	-		-	- +	
Rhodosporidium thermo-inf-atum	+	+	+	+	+	-	+
Rhodotorula glutinis	+	v	-		+	- v	+
R. graminis	+	+w or-	-		+	- -	+
R. minuta	+ or w	-	-		-	- -	+
R. rubra	+(w)	v	-		-	- (+w) v	+
Saccharomyces cerevisiae	- or +s	-	-		-	v v	
S. cairensis	-	-	-		-	- +w or-	
S. exiguus	- or +s	-	-		-	- or +w -	
S. telluris	- or +s	-	-		-	- +	
Saccharomycopsis lipolytica	+	+	-	-	-	- -	+
Sporidiobolus pararoseus	v	v	-		-	+ -	+
Sporobolomyces alba-ruberscens	+w	+w	-		-	- +w	+
S. pararoseus	-	-	+s		+	- -	+
S. roseus	v	v	-		+	v -	+
Stephanonseus ciferrii	+	+ or w	+	+	-	- +	
Torulaspora delbrueckii	-(+)	-	-		-	+s or - -	
T. globosa	-	-	-		-	+ +	
Trichosporon cutaneum	v	v	-	+	-	- v	+
Trich. pullulans	+	+	v	+	+	+ -	+
Wickerhamiella domergii	+	+	-		+	- -	
Zygosaccharomyces rouxii	-(+)	-	-		-	v v	

	Nitri	Sta form	30 C	25 C	50/50 gye	DBB col	10%NaCl
Cry. macrans		+	-	+	-		
cry. skinneri	-	+w	+w				
Cry. uniguttulatus	-	+ or w	v		-		
Debaryomyces castellii					+		
Debaryomyces hansenii							
De. marama	v			-	+		
De. polymorphus					w+		
Filobasidium uniguttulatum					+		
Hansenula californica					-		
Issatchenkia orientalis							
Leucosporidium scottii		-					
Metschnikowia pulcherrima		-			-		
Pichia carsonii					+w or -		
P. etchellsii							+
P. fermentans							+
P. guilliermondii							v
P. haplophiola							+
P. humboldtii							+s
P. media							-
P. membranaefaciens							+
P. rhodanensis							+ or s
Rhodosporidium infirmo-miniatum		+	+ or w		-		-
Rhodotorula glutinis		-			-		
R. graminis		-			-		
R. minuta		-			-		
R. rubra		-			-		
Saccharomyces cerevisiae				v			
S. dairensis				-			
S. exiguus				-			
S. telluris				-			
Saccharomycopsis lipolytica				-			
Sporidiobolus pararoseus	-	v		-			
Sporobolomyces alboruberescens	-	+		-			
S. pararoseus	+	-		-			
S. roseus	-	-		-			
Stephanoascus ciferrii				-			
Torulaspora delbrueckii				-			
T. globosa				+			
Trichosporon cutaneum				+w or -	+		
Tri. pullulans	+	-		-	+		
Wickerhamiella domergii						+	
Zygosaccharomyces rouxii				+			

II. Computer Program (MYID23 Su and Li, and MYID14 Su and LI)

```
(* This program is designed to identify meat yeast by biochemical tests. The data
of about 80 meat yeast stored under the file name-code.dat. The name of yeast
you identified and the test results will appear on screen and store on diskette
under a given file name.*)

program mycode(input,output,file 1,file 2);
const maxnum=200;
      blanks ='           ';
type str = array [1..12] of char;
     matrix = arry [1..maxnum,1.30] of char;
     namstr = arry [1..35] of char;
     list = array [1..maxnum] of namstr;
     tnm = array [1..3] of char;
tlist = array [1..maxnum] of tnm;
var coding : matrix;
    namlist : namstr;
    outfile : str;
    byt : tlist;
    filel,file2 : text;
(*****************************************************)
procedure gettest(var ntest : tlist; var tn : integer);
var k,l : integer;
begin
  k := l;
  while not eod(filel) do
   begin
    for l := l to 3 do
     begin
       read(filel,ntest[k,l]);
     end;
     en := k -l;
end;
(*****************************************************)
procedure getcode(var cd : matrix var lst : list;
                  var cn,bn : integer);
var k,l,m : integer;
begin
   k := l;
   while not eof(filel) do
    begin
      l := l;
      while not eoln(filel) do
      begin
       if (l >= l> and (l <= 35) then
           read(filel,namlist[k,l])
       else
           read(filel,cd[k,l-35]);
       l := l + l;
      end;
      readln(filel);
      k :=k + l;
     end;
```

```
    if eoln(file1) then
       cn := 1 - 1;
    if eof(file1) then
      bn := k - 1;
      writeln('en',en : 4, 'bn=', bn : 4);
end;
(*****************************************************)
procedure ident_code(var namlist : list; var coding : matrix;
                     var ntest : tlist; tn, bn,cn : integer);
type temp = array [1..23] of char;
var k,l,p,z,count,ctx,pnum : integer;
    ch : char;
    chk : temp;
    ok : boolean;
begin
    writeln(file2,'********** No. ', num : 5,'.'********** ');
    writeln('No. ', num : 5, '.','  ');
    writeln;
    for l := 1 to tn do
      begin
        write('Enter ');
        for p := 1 to 3 do
         write(ntest[l,p]);
       write(' test (+,-, or v): ');
       read(ch);
       writeln;
       if (ch = '+') or (ch = '-') or (ch ='v') then
       ynam[l] := ch
       else
       begin
        write('please reenter +.-,or v:,);
        readln(ch);
       end;
       write;
     end;

ctx := 1;
for z :=1 to bn do
 begin
   count := 1;
   pnum := 1;
   for l := 1 to tn do
    begin
     if (coding[z,l] = ynam[l] then
       begin
       ok := true;
       pnum : pnum + 1;
       end
       else
        if (coding[z,l] = 'v') then
        ok := true
        else
```

```
          begin
            ok := false;
            count := count + 1;
          end;
        end;
        if count = 1 then
         begin
         ctx := 100;
         for l := 1 to 35 do
         begin
           write(file2,namlist[z,l]);
           write(namlist[z,l]);
         end;
           write(file2,'prob =' :20, <pnum-1)/tn : 6 : 3);
           write('prob =' : 20, (pnum-1)/tn : 6 : 3);
           writeln(file2);
           writeln;
           for k := a to tndo
           begin
            for l := 1 to 3 do
            begin
            write(file2,ntest[k,l]);
            write(tnest[k,l]);
            end;
           end;
            writeln(file2);
            writeln;
           for k := 1 to tn do
            begin
               write(file2,' ', ynam[k], ' ');
               write(' ', ynam[k], ' ');
            end;
            writeln(file2);
            writeln(file2);
            writeln;
            writeln;
       end;
      end;
      if ctx = 1 then
      begin
       writeln(file2);
       writeln;
       writeln(file2,'unknown yeast ???');
       writeln('unknown yeast ???');
      end;
end;
(*****************************************)
begin
   clrscr;
   writeln('    ********************************************');
   writeln('    *                                          *');
   writeln('    * Welcome to Meat Yeast Identification     *');
```

```
writeln('    *                  version 1.0                  *');
writeln('    *                                               *');
writeln('    *              by Haiping Su                    *');
writeln('    *       Kansas State University                 *');
writeln('    *                                               *');
writeln('    ************************************************');
sound(200);
nosound;
sound(120); sound(1000);
nosound;
sound(200); sound(500);
assign(filel,'tests.dat');
reset(filel);
sound(100);
gettest(ntest,tnum);
close(100);
sound(500);
assign(filel),'code.dat');
reset(filel);
sound(300);
getcode(coding,namlist,cnum,bnum);
clrscr;
getcode(coding,namlist,cnum,bnum);
clrscr;
nosound;
writeln('enter output file name: ');
outfile := blanks;
readln(outfile);
readln(outfile);
assign(file2,outfile0;
rewrite(file2);
num :=l;
byt := ' ';
while byt <> 'n' do
begin
ident-code(namlist,coding,ntest,tnum,bnum,cnum);
num := num + l;
writeln('continue test ? <y or n>:');
readln(byt);
end;
close(filel);
close(file2);
clrscr;
writeln('    ************************************************');
writeln('    *                                               *');
writeln('    *              Thank You                         *');
writeln('    *                                               *');
writeln('    *             See You Again                      *');
writeln('    *                                               *');
writeln('    ************************************************');
sound(400);
nosound;
```

```
    sound(900);
    sound(200);
    nosound;
    sound(800);
    nosound;
end;
```

III. Output File for Data Interpretation by Using MYID14 for Named Yeast Cultures and Fresh Isolates:
(1) For named yeast cultures:

```
********** test #   1(C-3)**********

Candida              albicans                    Prob = 0.786   #of v= 3
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  -  -  -  -  +  -  -  -  -  -  +
Saccharomyces        cerevisiae                  Prob = 0.571   #of v= 6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  -  -  -  -  +  -  -  -  -  -  +
********** test #   2(I-7)**********

Candida              albicans                    Prob = 0.786   #of v= 3
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  -  -  -  -  +  -  -  -  -  -  +
Saccharomyces        cerevisiae                  Prob = 0.571   #of v= 6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  -  -  -  -  +  -  -  -  -  -  +
********** test #   3(I-8)**********

Candida              albicans                    Prob = 0.786   #of v= 3
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  -  -  -  -  +  -  -  -  -  -  +
Saccharomyces        cerevisiae                  Prob = 0.571   #of v= 6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  -  -  -  -  +  -  -  -  -  -  +
********** test #   4(C-6)**********

Candida              versatilis                  Prob = 0.643   #of v= 5
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  +  -  -  +  +  +  -  -  -  -
Sporobolomyces       roseus                      Prob = 0.714   #of v= 4

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  +  -  -  +  +  +  -  -  -  -
********** test #   5(C-14)**********


Unknown yeast ???
********** test #   6(I-13)**********

Candida              albicans                    Prob = 0.786   #of v= 3
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  -  -  -  -  +  -  +  +  -  -  +
Candida              azyma                       Prob = 0.929   #of v=
1
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  -  -  -  -  +  -  +  +  -  -  +
Candida              parasilosis                 Prob = 0.786   #of v= 3
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
```

```
 +  +  +  -  -  -  -  +  -  +  +  -  -  +
Candida              tropicalis                    Prob = 0.714   #of v=  4
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  -  -  -  -  +  -  +  +  -  -  +
****************** test #   7(N-1)**********************

Candida              valida                        Prob = 0.929   #of v=  1
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -
Candida              vini                          Prob = 0.929   #of v=  1
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -
Pichia               membranaefaciens               Prob = 0.786   #of v=  3

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -
Saccharomyces        cerevisiae                    Prob = 0.571   #of v=  6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -
****************** test #   8(C-27)**********************

Candida              valida                        Prob = 0.929   #of v=  1
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -
Candida              vini                          Prob = 0.929   #of v=  1
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -
Pichia               membranaefaciens               Prob = 0.786   #of v=  3

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -
Saccharomyces        cerevisiae                    Prob = 0.571   #of v=  6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -
****************** test #   9(N-14)**********************

Wickerhamiella       domergii                      Prob = 0.929   #of v=  1
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  -  -  +  -  -  -  -  -  +  +  -  -
****************** test #  10 (N-19)**********************

Candida              curvata                       Prob = 0.714   #of v=  4

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  +  +  +  +  +  +  -  +  +  -
Candida              humicola                      Prob = 0.429   #of v=  8

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  +  +  +  +  +  +  -  +  +  -
Cryptococcus         laurentii                     Prob = 0.857   #of v=  2
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
```

```
 +  +  +  +  +  +  +  +  +  +  -  +  +  -
Trichosporon          cutaneum                          Prob = 0.500   #of v=  7


GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  +  +  +  +  +  +  -  +  +  -
********** test #  11 (D-1)**********

Debaryomyces          hansenii                          Prob = 0.643   #of v=  5

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  +  -  -  -  +  +  +  -  -  +
Rhodotorula           rubta                             Prob = 0.429   #of v=  8

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  +  -  -  -  +  +  +  -  -  +
********** test #  12 (D-2)**********

Candida               famata                            Prob = 0.643   #of v=  5

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  -  -  -  +  +  +  +  -  -  -
Rhodotorula           glutinis                          Prob = 0.643   #of v=  5
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  -  -  -  +  +  +  +  -  -  -
********** test #  13 (D-3)**********

Debaryomyces          hansenii                          Prob = 0.643   #of v=  5

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  +  -  -  -  +  +  +  +  -  -
Rhodotorula           rubta                             Prob = 0.429   #of v=  8

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  +  -  -  -  +  +  +  +  -  -
********** test #  14 (N-5)**********

Candida               famata                            Prob = 0.643   #of v=  5

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  +  -  +  +  +  +  +  +  -  -
Candida               fenniea                           Prob = 0.786   #of v=  3

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  +  -  +  +  +  +  +  +  -  -
Candida               humicola                          Prob = 0.429   #of v=  8

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  +  -  +  +  +  +  +  +  -  -
Candida               membranaefaciens                  Prob = 0.786   #of v=  3

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
```

```
 +  +  +  +  +  -  +  +  +  +  +  +  -  -
Debaryomyces          marama                              Prob = 0.929   #of v=
1
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C

 +  +  +  +  +  -  +  +  +  +  +  +  -  -
Debaryomyces          polymorphus                         Prob = 0.857   #of v=2

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  +  -  +  +  +  +  +  +  -  -
******************** test #  15(N-24)**********************

Candida               famata                              Prob = 0.643   #of v=  5

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  +  -  +  +  +  +  +  +  -  -
Candida               fenniea                             Prob = 0.786   #of v=  3

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  +  -  +  +  +  +  +  +  -  -
Candida               humicola                            Prob = 0.429   #of v=  8

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  +  -  +  +  +  +  +  +  -  -
Candida               membranaefaciens                    Prob = 0.786   #of v=  3

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  +  -  +  +  +  +  +  +  -  -
Debaryomyces          marama                              Prob = 0.929   #of v=
1
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  +  -  +  +  +  +  +  +  -  -
Debaryomyces          polymorphus                         Prob = 0.857   #of v=
2
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  +  -  +  +  +  +  +  +  -  -
******************** test #  16(N-8)**********************

Candida               tropicalis                          Prob = 0.714   #of v=  4
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  -  -  -  +  -  +  +  +  -  +
Pichia                etchellsii                          Prob = 1.000   #of v=  0
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  -  -  -  +  -  +  +  +  -  +
******************** test #  17(N-9)**********************


Unknown yeast ???
******************** test #  18(P-2)**********************

Pichia                membranaefaciens                    Prob = 0.786   #of v=  3
```

```
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  +
Saccharomyces        cerevisiae                    Prob = 0.571   #of v=  6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  +
Saccharomyces        telluris                      Prob = 1.000   #of v=  0


GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  +
****************** test #  19(I-20)**********************

Pichia               membranaefaciens              Prob = 0.786   #of v=  3

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  +
Saccharomyces        cerevisiae                    Prob = 0.571   #of v=  6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  +
Saccharomyces        telluris                      Prob = 1.000   #of v=  0
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  +
****************** test #  20(S-5)**********************

Saccharomyces        cerevisiae                    Prob = 0.571   #of v=  6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  -  -  -  -  -  -  -  -  -  -  -  +
****************** test #  21(I-24)**********************

Saccharomyces        cerevisiae                    Prob = 0.571   #of v=  6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  -  -  -  -  -  -  -  -  -  -  -  -


(2) For fresh isolates:

****************** test #   1 (GP1)**********************

Saccharomyces        cerevisiae                    Prob = 0.571   #of v=  6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  +  -  -  -  -  -  -  -  -  -  -  -  -  -
****************** test #   2(GP)**********************

Pichia               membranaefaciens              Prob = 0.786   #of v=  3

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  +
Saccharomyces        cerevisiae                    Prob = 0.571   #of v=  6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  +
Saccharomyces        telluris                      Prob = 1.000   #of v=  0
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
```

```
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  +
****************** test #   3(GP3)**********************

Saccharomyces        cerevisiae                          Prob = 0.571   #of v=  6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  +  -  -  -  -  -  -  -  -  -  -  -  -  -  -
****************** test #   4(GP4)**********************


Unknown yeast ???
****************** test #   5(GP5)**********************

Saccharomyces        cerevisiae                          Prob = 0.571   #of v=  6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  +  -  -  -  -  -  -  -  -  -  -  -  -  -  -
****************** test #   6(GP6)**********************

Candida              valida                              Prob = 0.929   #of v=  1
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -
Candida              vini                                Prob = 0.929   #of v=  1
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -
Pichia               membranaefaciens                    Prob = 0.786   #of v=  3

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -
Saccharomyces        cerevisiae                          Prob = 0.571   #of v=  6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -
****************** test #   7(GP7)**********************

Saccharomyces        cerevisiae                          Prob = 0.571   #of v=  6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  +  -  -  -  -  -  +  -  -  -  -  -  -  -  -
Torulaspora          delbrueckii                         Prob = 0.643   #of v=  5
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  +  -  -  -  -  -  +  -  -  -  -  -  -  -  -
****************** test #   8(GP8)**********************


Unknown yeast ???
****************** test #   9(GP9)**********************

Saccharomyces        cerevisiae                          Prob = 0.571   #of v=  6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  +  -  -  -  -  -  +  -  -  -  -  -  -  -  -
Torulaspora          delbrueckii                         Prob = 0.643   #of v=  5
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  +  -  -  -  -  -  +  -  -  -  -  -  -  -  -
****************** test #  10(GP10)*********************
```

```
Saccharomyces          cerevisiae                          Prob = 0.571   #of v=  6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  -  -  -  -  -  -  +  -  -  -  -  -  -  -
Torulaspora            delbrueckii                         Prob = 0.643   #of v=  5
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  -  -  -  -  -  -  +  -  -  -  -  -  -  -
******************** test #  11(HD1)**********************

Saccharomyces          cerevisiae                          Prob = 0.571   #of v=  6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C

 -  -  -  -  -  -  -  -  +  -  -  -  -  -  -  -
Torulaspora            delbrueckii                         Prob = 0.643   #of v=  5
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  +  -  -  -  -  -  -  -
******************** test #  12(HD2)**********************

Saccharomyces          cerevisiae                          Prob = 0.571   #of v=  6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  +  -  -  -  -  -  -  -
Torulaspora            delbrueckii                         Prob = 0.643   #of v=  5
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  +  -  -  -  -  -  -  -
******************** test #  13(HD3)**********************

Candida                valida                              Prob = 0.929   #of v=  1
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -
Candida                vini                                Prob = 0.929   #of v=  1
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -
Pichia                 membranaefaciens                    Prob = 0.786   #of v=  3

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -
Saccharomyces          cerevisiae                          Prob = 0.571   #of v=  6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -
******************** test #  14(HD4)**********************

Saccharomyces          cerevisiae                          Prob = 0.571   #of v=  6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  +  -  -  -  -  -  -  -
Torulaspora            delbrueckii                         Prob = 0.643   #of v=  5
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  +  -  -  -  -  -  -  -
******************** test #  15(HD5)**********************

Saccharomyces          cerevisiae                          Prob = 0.571   #of v=  6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  +  -  -  -  -  -  +  -  -  -  -  -  -  -
Torulaspora            delbrueckii                         Prob = 0.643   #of v=  5
```

```
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  +  -  -  -  -  -  +  -  -  -  -  -  -  -
********** test #  16(HD6)**********

Saccharomyces          cerevisiae                    Prob = 0.571   #of v=  6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  +  +  -  -  -  -  -  +  -  -  -  -  -  -  -
Torulaspora            delbrueckii                   Prob = 0.643   #of v=  5
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  +  +  -  -  -  -  -  +  -  -  -  -  -  -  -
********** test #  17(HD7)**********


Saccharomyces          cerevisiae                    Prob = 0.571   #of v=  6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  +  +  -  -  -  -  -  -  -  -  -  -  -  -  -
********** test #  18(HD8)**********

Candida                valida                        Prob = 0.929   #of v=  1
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -
Candida                vini                          Prob = 0.929   #of v=  1
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -
Pichia                 membranaefaciens              Prob = 0.786   #of v=  3

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -
Saccharomyces          cerevisiae                    Prob = 0.571   #of v=  6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -
********** test #  19(HD9)**********

Saccharomyces          cerevisiae                    Prob = 0.571   #of v=  6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  +  -  -  -  -  -  -  +  -  -  -  -  -  -  -
Torulaspora            delbrueckii                   Prob = 0.643   #of v=  5
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  +  -  -  -  -  -  -  +  -  -  -  -  -  -  -
********** test #  20(HD10)**********

Saccharomyces          cerevisiae                    Prob = 0.571   #of v=  6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  +  -  -  -  -  -  -  +  -  -  -  -  -  -  -
Torulaspora            delbrueckii                   Prob = 0.643   #of v=  5
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  +  -  -  -  -  -  -  +  -  -  -  -  -  -  -
********** test #  21(HD11)**********

Candida                valida                        Prob = 0.929   #of v=  1
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -
```

```
Candida              vini                          Prob = 0.929   #of v= 1
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -
Pichia               membranaefaciens              Prob = 0.786   #of v= 3

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -
Saccharomyces        cerevisiae                    Prob = 0.571   #of v= 6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -
******************** test #  22(BO1)**********************

Candida              famata                        Prob = 0.643   #of v= 5


GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  -  -  -  +  +  +  +  -  -  -
Rhodotorula          glutinis                      Prob = 0.643   #of v= 5
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  -  -  -  +  +  +  +  -  -  -
******************** test #  23(BO2)**********************


Unknown yeast ???
******************** test #  24(BO3)**********************

Candida              versatilis                    Prob = 0.643   #of v= 5
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  -  -  -  +  +  -  -  -  -  -
Sporidiobolus        paraoseus                     Prob = 0.786   #of v= 3
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  -  -  -  +  +  -  -  -  -  -
Sporobolomyces       roseus                        Prob = 0.714   #of v= 4

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  -  -  -  +  +  -  -  -  -  -
******************** test #  25(BO4)**********************

Candida              versatilis                    Prob = 0.643   #of v= 5
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  -  -  -  +  +  +  -  -  -  -
Sporobolomyces       roseus                        Prob = 0.714   #of v= 4

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  -  -  -  +  +  +  -  -  -  -
******************** test #  26(BO5)**********************

Saccharomyces        cerevisiae                    Prob = 0.571   #of v= 6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  -  -  -  -  -  +  -  -  -  -  -
******************** test #  27(BO6)**********************
```

```
Candida              versatilis                    Prob = 0.643   #of v= 5
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  -  -  -  +  +  +  -  -  -  -
Sporobolomyces       roseus                        Prob = 0.714   #of v= 4

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  -  -  -  +  +  +  -  -  -  -
****************** test #  28(BO7)**********************

Candida              versatilis                    Prob = 0.643   #of v= 5
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  -  -  -  +  +  +  -  -  -  -
Sporobolomyces       roseus                        Prob = 0.714   #of v= 4

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  -  -  -  +  +  +  -  -  -  -

****************** test #  29(BO8)**********************

Candida              versatilis                    Prob = 0.643   #of v= 5
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  -  -  -  +  +  +  -  -  -  -
Sporobolomyces       roseus                        Prob = 0.714   #of v= 4

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  -  -  -  +  +  +  -  -  -  -
****************** test #  30(BO9)**********************

Candida              versatilis                    Prob = 0.643   #of v= 5
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  -  -  -  +  +  +  -  -  -  -
Sporobolomyces       roseus                        Prob = 0.714   #of v= 4

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  -  -  -  +  +  +  -  -  -  -
****************** test #  31(FP1)**********************

Candida              famata                        Prob = 0.643   #of v= 5

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  -  -  +  +  +  +  +  -  -  -
****************** test #  32(FP2)**********************

Candida              famata                        Prob = 0.643   #of v= 5

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  -  -  +  +  +  +  +  -  -  -
****************** test #  33(FP3)**********************

Candida              famata                        Prob = 0.643   #of v= 5

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
```

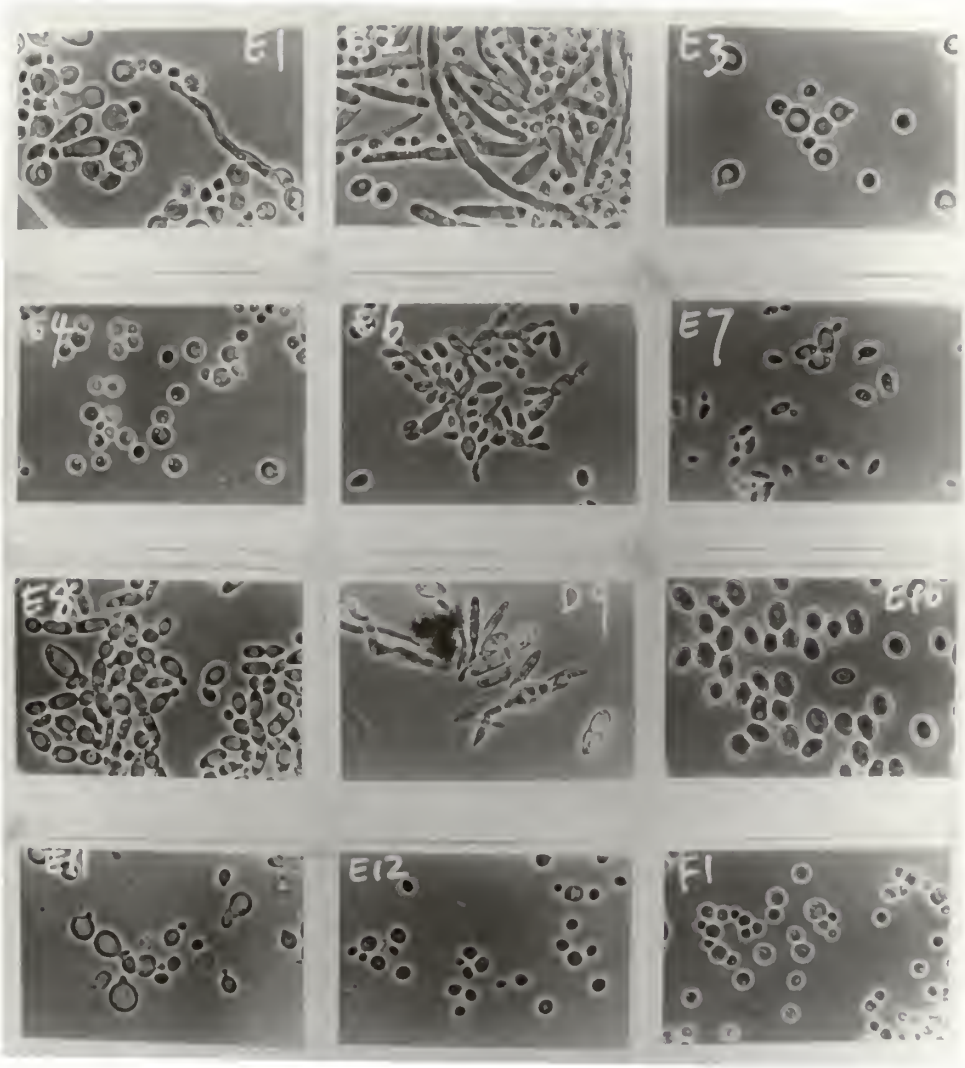
```
 +  +  +  +  -  -  +  +  +  +  +  -  -  -
******************** test #  34(FP4)**********************

Candida              famata                          Prob = 0.643   #of v=  5

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  -  -  +  +  +  +  +  -  -  -
******************** test #  35(FP5)**********************

Candida              famata                          Prob = 0.643   #of v=  5

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  -  -  +  +  +  +  +  -  -  -
******************** test #  36(FP6)**********************

Candida              famata                          Prob = 0.643   #of v=  5

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C

 +  +  +  +  -  -  +  +  +  +  +  -  -  -
******************** test #  37(FP7)**********************

Candida              famata                          Prob = 0.643   #of v=  5

GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  +  -  -  +  +  +  +  +  -  -  -
******************** test #  38(FP8)**********************


Unknown yeast ???
******************** test #  39(PE1)**********************

Candida              versatilis                      Prob = 0.643   #of v=  5
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  -  -  -  -  +  -  -  -  -  -  -  -
Saccharomyces        cerevisiae                      Prob = 0.571   #of v=  6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  -  -  -  -  +  -  -  -  -  -  -  -
Torulaspora          delbrueckii                     Prob = 0.643   #of v=  5
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 +  +  +  -  -  -  -  +  -  -  -  -  -  -  -
******************** test #  40(PE2)**********************

Saccharomyces        cerevisiae                      Prob = 0.571   #of v=  6
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  +  -  -  -  -  -  +  -  -  -  -  -  -  -
Torulaspora          delbrueckii                     Prob = 0.643   #of v=  5
GalSucMalCelRibRhaEryTreRafAraSAdCAdIno37C
 -  +  -  -  -  -  -  +  -  -  -  -  -  -  -
******************** test #  41(CH1)**********************
```

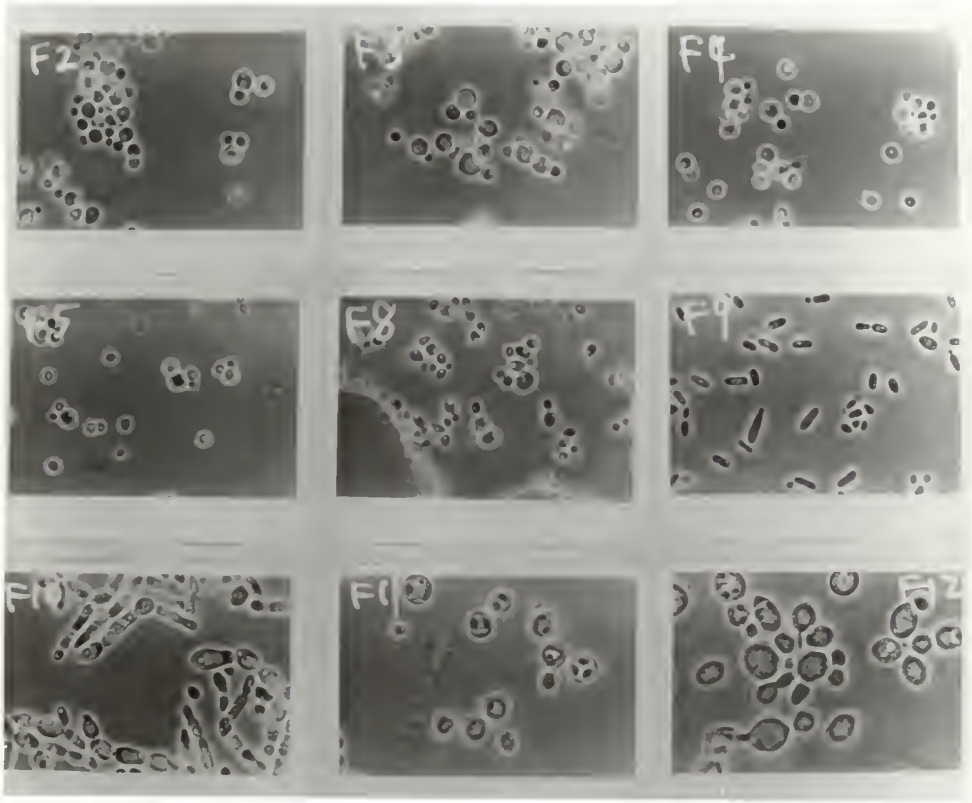
Unknown yeast ???

IV. Photomicrograph (phase contrast) for Named Yeast Cultures

All figures are magnified 1000 times.
All the cultures were grown on YM agar at 25C for 10 days.

Yeasts:	Stock code	picture #
Candida albicans	C-3	E 1
	I-7	E 2
	I-8	E 3
Candida famata	C-6	E 4
Candida parasilopsis	I-13	E 6
	C-14	E 11
Candida valida	N-1	E 7
Candida vini	C-27	E 8
Cryptococcus laurentii	N-19	E 12
Debaryomyces hansenii	D-1	F 1
	D-2	F 2
	D-3	F 3
De. marama	N-5	F 4
	N-24	F 5
Pichia etchellsii	N-8	E 10
P. media	N-9	F 8
P. membranaefaciens	P-2	F 9
	I-20	F 10
S. cerevisiae	S 5	F 11
	I-24	F 12
Wick. domercgii	N-14	D 9

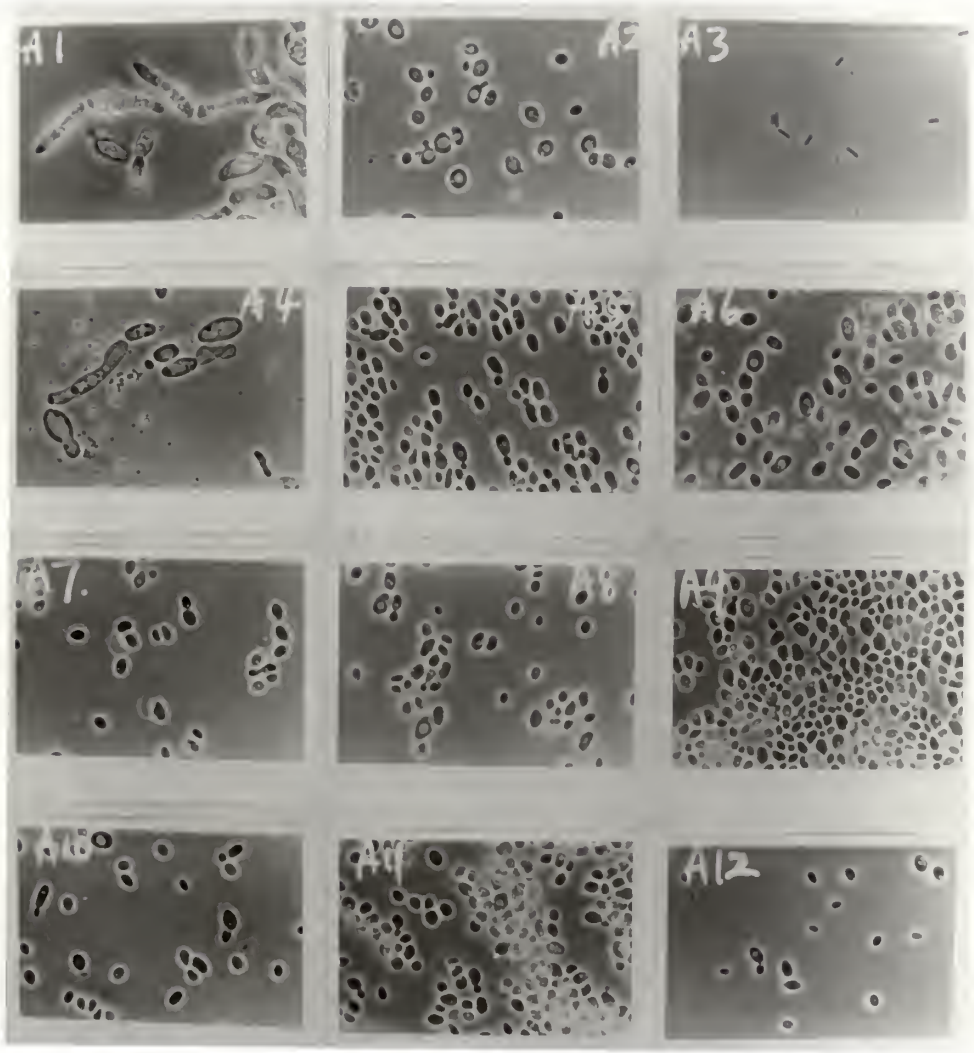

E1
E3
E7
E12
F1

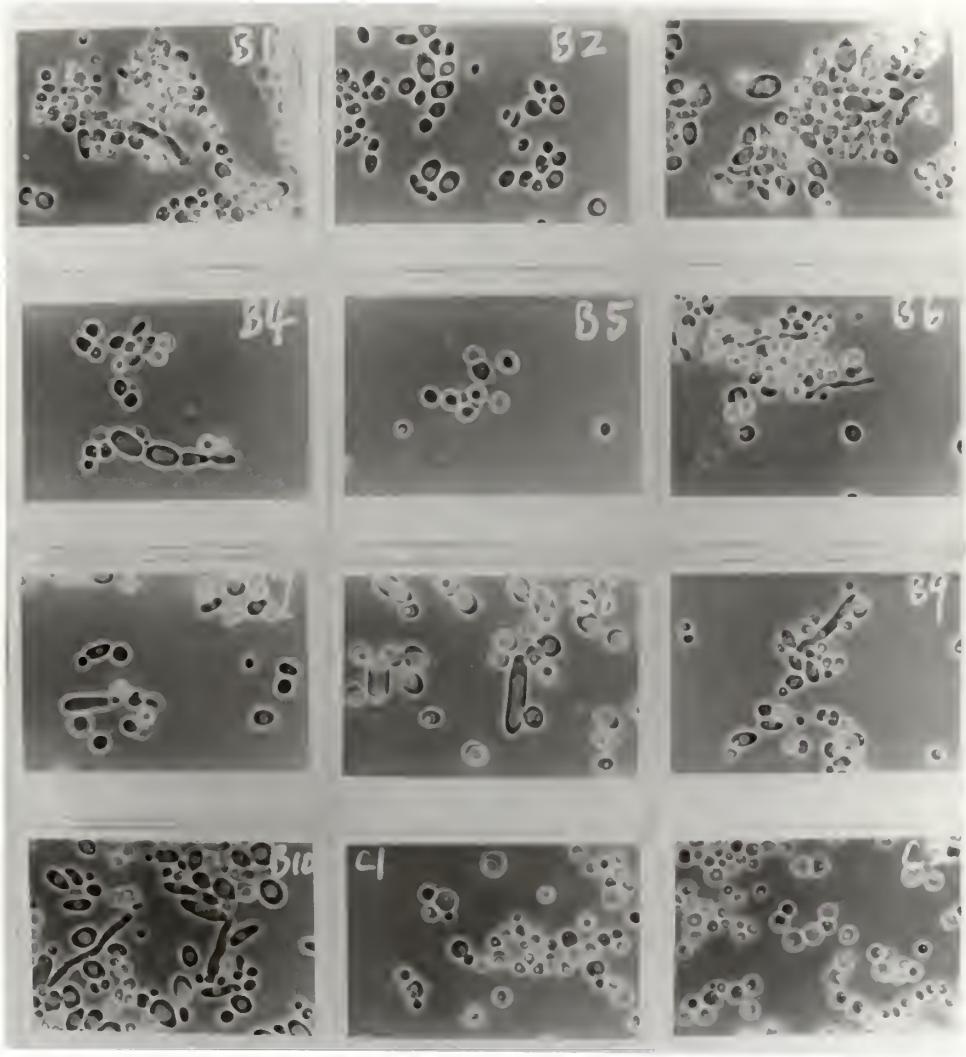

F2
F3
F4
F5
F8
F9
F10
F11
F12

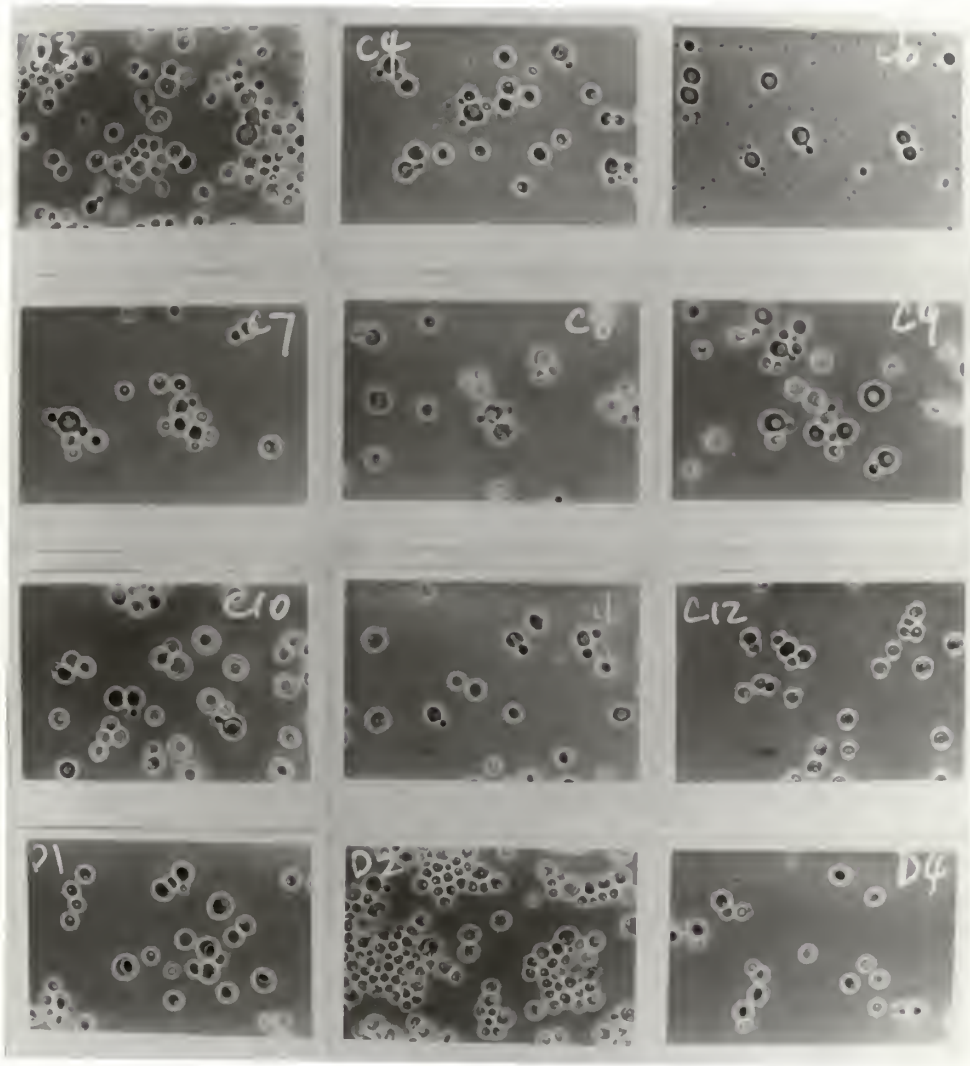
V. Photomicrograph (phase contrast) for Fresh Yeast Isolates from Meat Products:

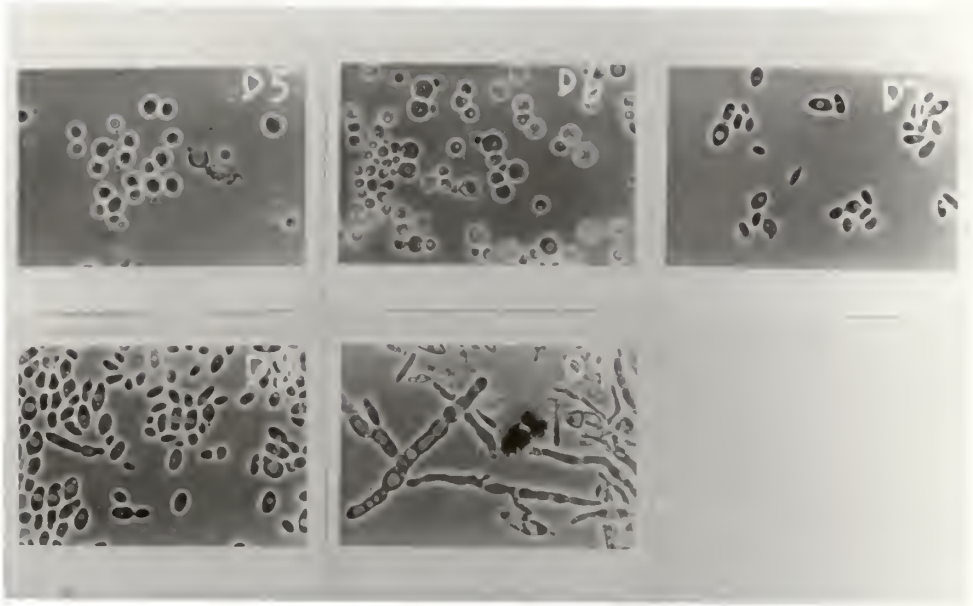
All the figures are magnified 1000 times.
All the cultures were grown on YM agar at 25C for 10 days.

Meat samples	Picture #
Ground pork	A 1, A 2, A 3, A 4, A 5, A 6, A 7, A 8, A 9, A 10.
Hot dog	A 11, A 12, B 1, B 2, B 3, B 4, B 5 B 6, B 7, B 9, B 10.
Bolonga	C 1, C 2, C 3, C 4, C 6, C 7, C 8, C 9, C 9, C 10.
Fresh pork	C 11, C 12, D 1, D 2, D 3, D 4, D 5, D 6.
Pepperoni	D 7, D 9.
Chicken	D 9.


A1
A2
A3
A4
A7
A12


B1
B2
B4
B5
B6
B10
C1


C4
C7
C9
C10
C12
D1
D2
D4



RAPID PRESUMPTIVE IDENTIFICATION OF YEAST IN MEAT PRODUCTS

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AN ABSTRACT OF THESIS

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ABSTRACT

In order to give a reliable discrimination of the wide range of species of yeast in food systems with less labor, material and time, two approaches for rapid presumptive identification of yeast in meat products were proposed: development of new dye-containing media and computer assisted identification system.

By screening 20 triarylmethane dyes against 42 species of yeast at 1:10,000 dilution in YM agar, two unique media for the presumptive identification of C. albicans and C. lipolytica were found. On acid blue 22 (Aniline blue) containing YM agar, C. albicans could fluoresce under long wave UV light (366nm) after 24 hour incubation at 21 C. C. pulcherrima fluoresced faintly after 48 hour of incubation. On basic blue 3 (Crystal violet, Flexo violet 615, and Flexo violet 600) containing YM agar, only C. lipolytica could grow.

Bacteria could not grow on antibiotics containing YM agar, and did not fluoresce on Aniline blue containing YM agar. On Aniline blue plus antibiotics containing YM agar Candida albicans fluoresced brightly after 24 hour incubation at 21 C while Candida lipolytica fluoresced weakly after 48 hour at 21 C.

The comparative study of performing 23 physiological tests by using Fung's Mini method and conventional method showed that both methods gave comparable results. However,

Fung's mini method only needed 2 to 3 days to obtain the expected positive results while the conventional method needed 10 to 15 days. The Fung's mini system also saved labor, material and space.

A simplified identification key (SIK), which consisted of 23 physiological tests and has a database for 84 species of yeast in meat products, was proposed. Two computer programs (MYID23 and MYID14) were developed to help interpret the results. MYID23 (Su/Li) could interpret 23 physiological tests and MYID14 (Su/Li) could interpret 14 physiological tests.

In the experimentation of SIK, the results showed that MYID14 was more practical than MYID23. Eighteen out of 21 named yeast cultures were correctly identified by MYID14 computer program.

The presumptive identification of 41 fresh yeast isolates from meat products by using MYID14 computer program and photomicrograph showed that various meat products had different yeast flora. The presumptive identification of yeasts by MYID14 computer program were: Candida famata, C. valida, C. versatilis, Saccharomyces cerevisiae and Torulopsis delbrueckii.

Photomicrographs for 21 named yeast cultures and 41 fresh yeast isolates from various meat products were used for further morphological confirmation of the presumptive identification of food-borne yeasts.